Casein and Caseinate Salts

CAS: [9000-71-9]

DESCRIPTION

Casein is an off-white to cream-colored granular or fine powder derived from the coagulum formed by treating skim milk with a food-grade acid (acid Casein), enzyme (rennet Casein), or other food-grade precipitating agent. After the precipitation, Casein is separated from the soluble milk fraction, washed, and dried. Chemically, Casein is a mixture of at least 20 electrophoretically distinct phosphoproteins. The main fractions—designated α-casein, β-casein, and κ-casein—are known to be mixtures, rather than single proteins. Casein contains all the amino acids known to be essential for human nutrition. It is insoluble in water and alcohol, but can be dissolved by aqueous alkalies to form Caseinate Salts. Caseinate Salts are white- to cream-colored granules or powders soluble or dispersible in water. They are prepared by treatment of Casein with food-grade alkalies, neutralizing agents, enzymes, buffers, or sequestrants. Common counter ions are NH_4^+, Ca^{++}, Mg^{++}, K^+, and Na^+.

Functional Use in Foods Binder; extender; clarifying agent; emulsifier; stabilizer.

REQUIREMENTS

Assay Not less than 90.0% protein for acid Casein; not less than 86.0% protein for rennet Casein; not less than 84.0% for Caseinate Salts, calculated on the dried basis.
Fat Not more than 2.25%.
Free Acid Passes test.
Heavy Metals (as Pb) Not more than 0.002%.
Lactose Not more than 2.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 12.0%.
Microbial Limits:
Aerobic Plate Count Not more than 100,000 per g.
Coliforms Not more than 2/0.1 g.
Salmonella Negative in 25 g.

TESTS

Assay Proceed as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein (P) by the formula

$$P = N \times 6.38,$$

in which N is the percent nitrogen.
Fat Transfer to a fat-extraction flask 1 g, accurately weighed; add 10 mL of water, and shake until homogeneous (warm if necessary). Add approximately 1 mL of ammonium hydroxide and heat in a water bath for 15 min at 60° to 70°, shaking occasionally. Add 10 mL of alcohol and mix well. Add 25 mL of peroxide-free ether, stopper, and shake vigorously for 1 min; allow to cool if necessary; add 25 mL of petroleum ether and repeat vigorous shaking. Allow the layers to separate and clarify or centrifuge at 600 rpm to expedite the process. Decant the

organic layer into a suitable flask or dish and repeat the extraction twice with 15 mL each of ether and petroleum ether for each extraction. Evaporate the combined ether extractions on a steam bath and dry the residue to a constant weight at 102°, or 70° to 75° at less than 50 mm Hg. Calculate the percent fat (F) by the formula

$$F = (R \times 100)/S,$$

in which R is the weight of the residue and S is the weight of the sample.

Free Acid Accurately weigh a 10-g portion of the finely ground sample, and transfer to a 500-mL conical flask. Add 200 mL of freshly boiled water maintained at 60°, swirl, and stopper. Place the flask in an 80° water bath, and hold for 30 min. Shake at 10-min intervals. Cool to room temperature, and filter. Accurately transfer a 100.0-mL portion of the clear filtrate to a 250-mL conical flask, add 0.5 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 30 s. Not more than 2.7 mL of 0.1 *N* sodium hydroxide is consumed.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lactose

Apparatus Use a suitable absorption spectrophotometer capable of operating in the visible range.

Phenol Reagent Heat a mixture of 8 g of phenol and 2 g of water until the crystals dissolve.

Lactose Solution Transfer approximately 2 g of lactose monohydrate, accurately weighed, to a 100-mL volumetric flask; dissolve in and dilute to volume with water.

Sample Solution Transfer approximately 1 g of sample, accurately weighed, to a 150-mL beaker. If the sample is acid Casein, add 0.10 g of sodium hydrogen carbonate. If the sample is rennet Casein, add 0.10 g sodium tripolyphosphate. Add 25 mL of water, and dissolve the sample by gently swirling while warming to 60° to 70° on a hot plate. Cool the solution to ambient temperature and add 15 mL water, 8 mL of 0.1 *N* hydrochloric acid, and 1 mL of a 10% solution of acetic acid. Mix well by swirling, and after 5 min, add 1 mL of 1 *M* sodium acetate; mix well.

After the precipitate has settled, filter and discard the first 5 mL of filtrate. Pipet 2 mL of the remaining filtrate into a test tube, add 0.2 mL *Phenol Reagent*, and mix well. Add 5 mL of sulfuric acid using an automatic dispenser or other means that permits mixing within 1 to 2 s. Ensure that the solution has been thoroughly mixed, and allow it to stand for 15 min, then cool to 20° in a water bath for 5 min.

Standard Solutions Transfer 10 mL of *Lactose Solution* to a 100-mL volumetric flask; dissolve in and dilute to volume with water (diluted *Lactose Solution*). Transfer respectively, 1, 2, 3, and 4 mL of diluted *Lactose Solution* to four 100-mL volumetric flasks; dilute to volume with water. These dilutions (standard dilutions) contain 20, 40, 60, and 80 µg of lactose per mL of solution, respectively. Into each of five test tubes add, in sequence, 2 mL of water and, respectively, 3 mL each of the standard dilutions of lactose. Then to each test tube add *Phenol Reagent* and sulfuric acid as described under *Sample Solution*.

Calibration Determine the absorbance of each *Standard Solution* at 490 nm against the water blank. Calculate the slope of the curve obtained by plotting absorbance versus μg/mL of lactose. The slope of the curve is the absorptivity (a) of the lactose–reagent product, presuming a cell of 1-cm pathlength is used for absorbance readings.

Procedure Determine the absorbance of the *Sample Solution* at 490 nm against a blank prepared using identical reagents.

Calculation Calculate the percent lactose (L) in the Casein sample by the formula

$$L = (A \times 0.00475)/(a \times m),$$

in which A is the absorbance of the *Sample Solution* at 490 nm; a is the absorptivity calculated under *Calibration*; m is the sample weight, in g; and the numerical factor accounts for dilution and conversion to percent from μg/mL.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 μg of lead ion in the control.

Loss on Drying, Appendix IIC Dry at 102° for 3 h.

Microbial Limits:

Aerobic Plate Count Proceed as directed in chapter 3, *FDA Bacteriological Analytical Manual*, Seventh Edition, Food and Drug Administration, 1992.

Coliforms Proceed as directed in chapter 4, *FDA Bacteriological Analytical Manual*, Seventh Edition, Food and Drug Administration, 1992.

Salmonella Proceed as directed in chapter 5, *FDA Bacteriological Analytical Manual*, Seventh Edition, Food and Drug Administration, 1992.

Packaging and Storage Store in well-closed containers.