Methods of Analysis

Important: *In order to assure reliability of grading analysis, it is imperative that the sample be well mixed for uniformity. If the sample completely fills the container, or if a composite of two or more samples is desired, transfer to a larger, clean, dry, sterile container of ample size, to permit thorough stirring with a dry sterile spoon or other appropriate agitation. Avoid undue exposure to the air which might cause contamination or moisture absorption.*

When a large quantity of dry milk is to be sampled, it is recommended that for every 4000 pounds of product, or any fraction thereof, a minimum of one sample be taken. After determining the total number of samples required, the sample should be taken from units uniformly distributed throughout the lot in question.

Sampling

Dry milk products readily absorb moisture and take on foreign odors when exposed to them for even a short period of time. It is important that conditions of sampling prevent absorption of moisture and foreign odors. In addition, samples and product should be protected against dust or other contamination.

The following precautions should be carefully observed:

Sampling should be done in clean, dry surroundings, free from dust and foreign odors.

Do not sample in a humid atmosphere, in a damp, cold storage room, or in a room into which steam is being discharged.

Do not sample unless hands and clothes are clean and dry.

Do not sample when attention is being distracted by other duties.

All sample containers and utensils used in sampling must be clean, sterile, dry, and at room temperature.

Sterile containers shall be used. Flexible polyethylene bags are recommended.

Samples are best withdrawn from bags and other bulk containers by use of a stainless steel, dry milk trier. This is done by plunging the sterile trier down through the center of the opened container and withdrawing the sample. The slot in the trier affords an opportunity to examine for color uniformity and particle characteristics. Several portions may be withdrawn in this manner until the required amount of total sample is obtained. Eject the sample from the trier with the clean, sterile handle of a spoon or similar device.

Sampling (continued)

When a trier is not available, a sterile spoon may be used to withdraw the sample. All samples taken in this manner should be withdrawn at least 6" from any surface.

After withdrawing the sample, the original container should be carefully reclosed at once.

Close the sample container immediately after the sample has been collected and thoroughly blended.

To avoid damage when transporting or mailing, pack each sample container in corrugated cartons.

Composite Sampling

When an analysis is desired on a composite of two or more individual samples, the composite should be prepared with the following precautions:

The individual samples should not be less than 4 oz. (100g).

Do not composite more than six individual samples.

Prepare each composite sample in a container sufficiently large to facilitate thorough blending. Turn over the contents with a sterile dry spoon of suitable size by lifting from the lowest edge of the container, at the same time rotating the container while it is tipped at an angle of 45°. Mix in this manner for a period of 1min. Avoid absorption of moisture and contamination from dust during this process.

Determination of Standard Plate Count (SPC)

Introduction

The most commonly used microbiological count method is the standard plate count (SPC) agar method. This method is used by the dairy industry for estimating the microbial populations in most types of dairy products and samples. It is also for determining quality and sources of contamination at successive stages of processing. The SPC, which is the reference method recognized by the NCIMS and specified in the Grade A Pasteurized Milk Ordinance, estimates the microbial population (primarily mesophilic, aerobic, and facultative bacteria) of raw and pasteurized milk and milk products.

Procedures for determining SPC in dairy products are contained in *Standard Methods for the Examination of Dairy Products,* published by the American Public Health Association. The latest edition of this publication should be consulted and used as the reference for a more detailed discussion of apparatus, reagents, and essential bacteriological techniques. The following procedures are recommended to assist laboratories in performing the Standard Plate Count for dry milk samples.

Apparatus and Reagents:

Agar- Standard Methods Agar (Tryptone Glucose Yeast Agar).

Aluminum foil or paper - foil or hand-surfaced paper, cut into pieces and sterilized, for weighing sample.

Autoclave - of a suitable size and capacity to provide uniform chamber temperature up to and including 121°C.

Balance - Torsion, or similar type, of approximately 500 g capacity and 0.1 g or better sensitivity.

Beakers - stainless steel for reconstitution of dehydrated medium.

Bottles - dilution; 8 oz., wide-mouth, leak-proof; for initial, reconstituting blank.

Bottles - dilution; resistant glass with suitable closure. Should be indelibly marked at the 99 ml ± 2 ml level.

Bunsen burner

Colony counter - Quebec, or one providing equivalent magnification and visibility.

Cotton - non-absorbent, for plugging flasks in medium preparation and sterilization.

Flasks - heat-resistant Erlenmeyer, or equivalent, for sterilization of medium.

Incubator - thermostatically controlled to maintain a uniform, constant temperature of 32°C ± 1°C.

Oven - hot air, with temperature range 0-220°C.

Determination of SPC (continued)

Petri dishes - 15 x 100 mm, Pyrex or Kimax, with flat bottoms, free from bubbles, scratches and other defects; or, single-service plastic.

Pipettes - reusable glass, or single-service plastic; 1.0 and 1.1, 2.2, and 11.0 ml.

Protective containers - stainless steel, or other suitable material, to protect Petri dishes and pipettes from recontamination between sterilization and use.

Tally - mechanical hand or electronic counting device.

Water - phosphate buffered distilled.

Water bath - thermostatically controlled to hold melted medium at 44-46°C.

Preparation of Agar:

Prepare according to bottle label instructions and in accordance with *Standard Methods for the Examination of Dairy Products.*

Transfer the medium to Erlenmeyer flasks, of appropriate size, stopper with cotton plugs and sterilize by autoclaving. After sterilization, cool to solidification and place in storage. The pH reaction of the sterilized agar should be 7.0 ± 0.2. If the pH is outside of this range, it must be adjusted to within this range. The pH may be determined electrometrically or colorimetrically, at 25°C.

Preparation of Dilution Blanks:

Prepare 99 ml dilution blanks with phosphate buffered distilled water. A stock buffer solution is prepared by dissolving 34g KH_2PO_4 in 500 ml distilled water and adjusting pH to 7.2 with 1N NaOH before making up to 1 L. Use 1.25 ml of this stock solution and dilute to a volume of 1 L with distilled water in making the dilution blanks. *Each dilution blank, after sterilization by autoclaving, should contain 99ml ± 2 ml.*

Use Escher rubber stoppers, or screw caps, for dilution blank closures. Friction-fit liners in screw caps should be used, as necessary, to make the closure leak-proof. *Never use cotton plugs as dilution blank closures.*

Sterilization:

Sterilize agar medium and dilution banks by autoclaving at 121°C (15 lbs steam pressure) for 15 min.

Loosen stoppers on dilution bottles to permit free passage of steam during sterilization.

Determination of SPC (continued)

Exhaust chamber air before closing autoclave drain valve and raising the chamber pressure.

Determine sterilization temperature with an accurate thermometer.

Do not overload autoclave.

Sterilize Petri dishes, and other equipment that will not burn or char, in the hot air oven at a minimum of 170° C for not less than 1 hr – after allowing the temperature to reach 170°C. If the sterilizer is crowded, sterilize for an additional period of time.

Preparation of Sample:

For initial dilutions, adjust blanks to 45°C and promptly weigh 11 g of sample directly into wide-mouth dilution bottle containing 99 ml of sterile, phosphate buffered distilled water; or, using sterile spatula or spoon, weigh 11 g of sample onto sterile aluminum foil or hard-surfaced paper and immediately aseptically transfer contents to initial dilution blank.

In preparing initial dilutions, observe special precautions to completely dissolve dry milk to obtain a homogeneous mixture. Mildly agitate the dilution blank to completely wet the sample. Allow to soak 2 min; shake dilution bottle making 25 complete up-and-down movements of about 1 ft (30 cm) in 7 sec.

Prepare appropriate dilutions for plating.

Dilution and Plating:

Prepare and plate such dilutions that Petri dishes, after incubation, will show between 25 and 250 colonies. Experience will indicate the dilution(s) necessary to obtain satisfactory dishes.

Dilutions should be made as follows:

(a) 1:10 - prepared by transfer of 11g dry milk to a 99 ml sterile dilution blank. Plate 1.0 ml.

(b) 1:100 - transfer 11 ml of (a) (1:10 dilution) into a 99 ml sterile dilution blank. Plate 1.0 ml, or a 1:100 dilution may be obtained by plating 0.1 ml of (a).

(c) 1:1,000 - transfer 11 ml of (b) (1:100 dilution) into a 99ml sterile dilution blank. Plate 1.0 ml or, a 1:1,000 dilution may be obtained by plating 0.1 ml of (b).

(d) 1:10,000 - transfer 11 ml of (c) (1:1,000 dilution) into a 99 ml sterile dilution blank. Plate 1.0 ml or, a 1:10,000 dilution may be obtained by plating 0.1 ml of (c).

Determination of SPC (continued)

Higher dilutions are prepared similarly. Shake all dilution blanks 25 times in the space of 1 ft (30cm) in 7 sec before transferring any aliquots therefrom.

The same pipette may be used for all transfers from the same dilution, *provided the pipette has not become contaminated.* Select a fresh, sterile pipette for transfer from each new dilution blank, or if the pipette in use has become contaminated by contacting other than intended surfaces.

Follow precautions in handling pipettes and in measurement and delivery therefrom as detailed in *Standard Methods for the Examination of Dairy Products.*

Pour 10-12 ml liquefied sterile agar (melted and tempered to 44-46°C) into each dish. Do not depend upon the sense of touch as an index of the proper temperature of melted agar; rather, use a separate flask of 1.5% aqueous agar solution, into which a thermometer is immersed, as a temperature control of the medium in the water bath. Sterile agar should be held as a liquid for a limited period of time. If held in this condition too long, a precipitate may form, the pH may change and solidifying power be lessened, making it unsatisfactory for use. Do not return flasks of agar to storage after they have been partially used.

Thoroughly mix the sample and agar in the dish and evenly spread over the Petri dish bottom by carefully rotating and tilting the dish without spilling. Allow the agar to solidify. The interval between the initial reconstitution of the sample and pouring the medium should not exceed 20 min.

An overlay of 3-5 ml of sterile agar may be added as a cover-layer to solidified agar pour plates. This procedure may be effective in controlling spreading colonies that frequently are produced by aerobic, sporeforming bacteria.

Incubation and Counting Petri Dishes:

Incubate Petri dishes in an inverted position. Arrange stacks of dishes so that they are separated from each other, and from the top and walls of the incubator, by at least 1". Do not stagger stacks of dishes on successive shelves.

Incubate at 32°C for 48 hr ± 3 hr.

Count all visible colonies on the dishes, including pin-point colonies, using a colony counter and with the aid of a tally counter.

If spreaders occur, count each spreader as a colony. Discard dishes when spreaders cover more than half the dish.

If undissolved particles of dry milk may be encountered and confused with pin-point colonies, verify the identity of each under the low power of a microscope. Duplicate dishes, held under refrigeration for comparison with the incubated dishes, are recommended if undissolved particles are encountered.

Determination of SPC (continued)

Reporting the Bacterial Estimate:

The actual colony count is converted to the bacterial estimate (Standard Plate Count), per gram, by application of the proper dilution factor. Since plate colony counts are subject to variations inherent in bacterial growth and measurement, it is desirable to report counts in a manner that will eliminate false impressions of accuracy. Report estimate to two significant figures only, i.e., 193 colonies on a 1:1000 dilution is reported as 190,000.

When the only dishes available show more than 250 colonies, report as too numerous to count ("TNTC"), or the count as estimated in accordance with the methods prescribed in *Standard Methods for the Examination of Dairy Products.* When under 25 colonies appear per dish, report as "Less than" 25 times the corresponding dilution, i.e., 19 colonies on a 1:100 dilution is reported as "Less than 2,500 per gram".

Note: *Growth in agar plates does not represent the total number of bacteria present in the product; it merely indicates the number of colonies that have developed under specified conditions in the limited amount of sample used.*

Reference:

Standard Methods for the Examination of Dairy Products, current edition.

Bacterial tests for presence of coagulase-positive staphylococci and salmonella, when needed, shall be made by referring to appropriate methods described in the FDA BAM Manual, current edition.

Petri-Film is an alternative method. Before usage each laboratory should validate method performance against the standard method.

Determination of Coliform Bacteria

Introduction

The Coliform group of bacteria comprises all aerobic and facultatively anaerobic, gram-negative, non-spore forming rods that are able to ferment lactose with the production of acid and gas at 32º or 35ºC within 48 hours. (The temperature of incubation should be specified when reporting results.) One source of these organisms is the intestinal tract of warm-blooded animals. Certain bacteria of nonfecal origin are also members of this group. Typically, these organisms are classified in the genera *Escherichia, Enterobacter,* and *Klebsiella*. A few lactose-fermenting species of other genera are also included in the Coliform group. In proportion to the numbers present, the existence of any of these types in dairy products is suggestive of unsanitary conditions or practices during production, processing, or storage.

Application of the test for coliforms is not intended to detect fecal pollution, but to measure the quality of the practices used to ensure proper processing and to minimize bacterial contamination of processed dairy products.

Apparatus and Reagents:

Agar - Violet Red Bile.

Aluminum foil or paper - foil or hard-surfaced paper, cut into pieces and sterilized, for weighing sample.

Autoclave - of a suitable size and capacity to provide uniform chamber temperature up to and including 121°C.

Balance - Torsion, or similar type, of approximately 500 g capacity and 0.1 g or better sensitivity.

Bottles - dilution; 8 oz., wide-mouth, leak-proof; for initial, reconstituting blank.

Colony counter - Quebec, or one providing equivalent magnification and visibility.

Incubator - thermostatically controlled to maintain a uniform, constant temperature of 32°C ± 1°C.

Petri dishes - 15 x 100 mm, Pyrex or Kimax, with flat bottoms, free from bubbles, scratches and other defects; or single-service plastic.

Pipettes - reusable glass, or single-service plastic; 10 ml.

Water - phosphate buffered distilled.

Water bath - thermostatically controlled to hold melted medium at 44-46°C.

Determination of Coliform Bacteria (continued)

Procedure:

Weigh 11g of dry milk onto a sterile glazed paper and transfer into 99 ml of sterile phosphate buffered distilled water. Mildly agitate the dilution bland in order to completely wet the sample.

Hold for 1-3 min. and vigorously shake 25 times, up and down, through a distance of 1 ft (30cm) in 7 sec.

Prepare a 1:1 dilution by pipetting 10 ml of the 1:10 dilution and distribute into three Petri dishes. Add 15-20 ml of tempered Violet Red Bile Agar (44-46°C) to each dish and thoroughly mix contents. After agar has solidified, add an extra 3-4 ml of the agar into each dish so as to evenly cover the surface and allow to solidify.

Invert the Petri dishes and incubate for 24 hr ± 2 hr at 32°C ± 1°C.. Count dark red colonies at least 0.5 mm in diameter as coliform colonies. Combine the counts of the three dishes and report as coliforms per gram of dry milk. If no colonies are present, report as less than 1 per gram.

Reference:

Standard Methods for the Examination of Dairy Products, current edition.

Petri-Film is an alternative method. Before usage each laboratory should validate method performance against the standard method.

Determination of Moisture

(Vacuum Oven Method)

Apparatus and Reagents:

Balance – analytical.

Desiccator

Vacuum oven – with drying column.

Weighing bottles – 60 x 30 mm glass, with stoppers.

Weighing pans – metal, round, flat-bottomed, covered weighing pan, 5 cm in diameter

Procedure:

Accurately weigh 1 to 2 g sample into a round flat-bottomed metal dish. The dish should be 5 cm in diameter with a close fitting slip-on cover. Loosen cover and place dish directly on the metal shelf in a vacuum oven kept at the temperature of boiling water. Dry to constant weight (approximately 5 hours) under 26 inches (4" Hg) of vacuum. During drying, admit into the oven a slow current of air, about 2 bubbles per second, dried by passing through H_2SO_4 or a solid desiccant of indicating calcium sulfate. Discontinue action of vacuum pump and carefully admit dried air into the oven. Remove from oven, transfer the hot weighing dishes immediately to a desiccator with the top ajar. Once the dishes have cooled, open the desiccator and immediately press the covers onto the dishes to prevent the absorption of moisture and weigh. Calculate percent loss in weight as moisture.

Reference: *Standard Methods for the Examination of Dairy Products,* current edition.

(Mechanical Convection Oven Method)

Apparatus and Reagents:

Balance – analytical.

Desiccator

Convention oven – with drying column.

Weighing bottles – 60 x 30 mm glass, with stoppers.

Weighing pans – metal, round, flat-bottomed, covered weighing pan, 5 cm in diameter.

Determination of Moisture (continued)

Procedure:

Weigh a 10 g sample accurately to the third decimal place into a metal dish 5 cm in diameter with a close fitting slip-on cover. Place in a mechanical convection oven with the dish

cover set at a slant toward the air flow. Dry 16 hr at 100 – 103C. Remove from oven, transfer the hot weighing dishes immediately to a desiccator with the top ajar. Once the dishes have cooled, open the desiccator and immediately press the covers onto the dishes to prevent the absorption of moisture and weigh. Calculate the percent loss in weight as moisture.

In the event of a dispute or appeal, the moisture shall be determined by the Vacuum Oven Method.

***Note**: Quick methods, such as the use of a moisture balance incorporating infra-red heating and a variable transformer or other methods, may be used in dry milk plants for determining moisture. While such methods perform a useful function in processing control, they frequently yield erroneous results due to improper selection of temperature-time conditions for conducting the test. It is strongly recommended that before such a method is used, it should be carefully standardized against the vacuum or mechanical convection oven method and checked against one of the latter methods each day it is used. Where moisture tests are required for specification purposes, the vacuum or mechanical convection oven method shall be used.*

Reference:

USDA Method for determining moisture

Determination of Milkfat

(Mojonnier Method)

Apparatus and Reagents:

Alcohol – ethyl, S.D. #1 or 3-A.

Ammonium hydroxide (NH_4OH) – Specific Gravity 0.9

Balance – analytical.

Bath – steam.

Desiccator

Dish - weighing, aluminum, flat-bottom.

Ether – ethyl, purified for fat extraction, peroxide free.

Ether – petroleum, boiling range 30-60°C.

Ethyl alcohol, 95% - no residue upon evaporation

Ovens – convection or vacuum.

Phenolphthalein indicator – 0.5% (m/v) in ethyl alcohol

Stoppers - neoprene, No 1.

*Mojonnier Dairy Products Tester**- Model A, D, F, H, or equivalent.

*Mojonnier centrifuge basket**- Part No. T66, or equivalent.

*Mojonnier fat extraction flask** - Part No. G3, or equivalent.

Procedure:

1. Preparation of weighing dish –

   a. Place a clean weighing dish in a convection oven at 102°C ± 2°C for 1 hr or in a vacuum oven at 100°C under pressure below 50 mm Hg for 10 min and cool to room temperature in a desiccator.

   b. If a Dairy Tester is used, condition the clean weighing dish in the vacuum oven at 135°C for 5 min, then transfer to the cooling desiccator for 7 min.

2. Weigh 1-1.25 g of dry milk (to the nearest 1.0 mg) onto a piece of glazed paper and quantitatively transfer to a dry fat extraction flask.

* Mojonnier equipment available from Nelson-Jameson, Inc., P.O. Box 647, 2400 E. Fifth St., Marshfield, WI 54449 (715) 387-1151

Determination of Milkfat (continued)

3. Add 9.0 ml warm distilled water, stopper the flask, shade until sample is homogeneous, then cool to room temperature.

4. Add 1.5 ml ammonium hydroxide, stopper the tube and mix thoroughly, then add 10 ml of ethyl alcohol and mix thoroughly.

5. Add 3 drops of phenolphthalein indicator to help sharpen the visual appearance of the interface between the ether and aqueous layers during the extraction.

6. For the first extraction, add 10 ml of ethyl alcohol, stopper and shake the flask for 15 secs.
7. Add 25 ml ethyl ether, stopper the tube and shake vigorously for 1 min., releasing built-up pressure by loosening the cork as necessary.

8. Add 25 ml petroleum ether, stopper the flask and shake vigorously for 1 min.

9. Let the mixture stand until the ether layer is practically clear (about 20 min) or centrifuge at 600 rpm for approximately 1 min if a Dairy Tester is used. See Note.

10. Tare weigh a weighing dish from 1 and carefully decant as much of the ether layer as possible into it. Rinse the lip of the flask with a small amount of petroleum ether catching the rinsings in the dish.

11. Evaporate the ether on a steam bath or hot plate so that spattering or boiling is avoided.

12. Add 4-5 ml ethyl alcohol to the flask and mix. Repeat steps 6 through 11. (For dry whole milk, skim, and buttermilk repeat steps 6 – 11 a 3rd time.)

13. After the ether has been evaporated in step 10, dry the extracted fat to constant weight in a convection oven at 102°C ± 2°C or a vacuum oven at 70-75°C under pressure below 50 mm Hg, cool to room temperature in a desiccator and weigh to the nearest 0.1 mg.

14. Run a blank along with the samples and determine solvent residue weight. The residue weight should not be more than 0.5 mg. If residue weight is greater than 0.5 mg, replace or purify reagents.

Calculations: $$\frac{\%\ \text{Fat} = \text{Weight of fat (g)} - \text{Weight residue (g)}\ (100)}{\text{Sample Weight (g)}}$$

Note:

If the ether-aqueous interface is not clearly defined after standing, gently swirl the flask to break any emulsion formed.

References:

Standard Methods for the Examination of Dairy Products, current edition.

Determination of Protein

Reference:

Standard Methods for the Examination of Dairy Products, current edition.

Determination of Solubility Index

Apparatus and Reagents:

Balance – Torsion, or similar type, approximately 500 g capacity and 0.1 g or better sensitivity.

Centrifuge – of required rpm with cups to accommodate conical centrifuge tubes. Steam turbine or heated Babcock centrifuge should not be used. The required rpm should be determined with test samples and with the door of the centrifuge closed. The following table gives the required rpm for different diameters of the head.

10 inch diameter	1075 rpm	18 inch diameter	800 rpm
12 inch diameter	980 rpm	20 inch diameter	759 rpm
14 inch diameter	909 rpm	22 inch diameter	724 rpm
16 inch diameter	848 rpm	24 inch diameter	695 rpm

The diameter of the head is the distance between the inside bottoms of opposite cups measured through the center of rotation of the centrifuge head while the cups are horizontally extended.

Centrifuge tubes - conical, graduated as follows*:*

From 0 - 1.0 ml in 0.1 ml divisions,
From 1.0 - 2.0 ml in 0.2 ml divisions,
From 2.0 - 10.0 ml in 0.5 ml divisions,
From 10.0 - 20.0 ml in 1.0 ml divisions,
and a 50.0 ml mark at least one-half inch from the top of tube.

Defoaming agent – Antifoam B emulsion, A5757 (Sigma Chemical Co., St. Louis, MO).

Mixer – Waring, 7-speed commercial blender with glass container and blending assembly (Waring catalog #7012G; Waring model #31BL42).

Siphon tube – Glass (for siphoning milk from the centrifuge tubes).

Water – distilled.

Procedure:

Add the quantity of powder specified in Table 1 to 200 ml distilled water at the temperature specified in Table 1 in the glass mixing container, and add defoaming agent (2-3 drops).

Determination of Solubility Index (continued)

Table 1

Product Type	Powder (g)	Water Temperature (°F)	Mixing Time (Minutes)
Nonfat Dry Milk (NFDM), Skim Milk Powder (SMP)	20	75	1.5
Dry Buttermilk, Dry Buttermilk Product	20	75	1.5
Whey Powder, Whey Protein Concentrate (WPC), Whey Protein Isolate (WPI)	20	75	1.5
Whole Milk Powder (WMP)	26	75	1.5
Milk Protein Concentrate (MPC) <= 70% protein content	15	140	2
Milk Protein Concentrate (MPC) > 70% protein content, Milk Protein Isolate (MPI), Micellar Casein	8	140	10

Note: depending on the particular product application, a variation in the quantity, temperature, or mixing time listed in Table 1 may be appropriate to better represent required product functionality.

Place the glass container on the mixer and mix for no less than the time specified in Table 1, at the #1 (3,000-3,500 rpm) speed setting of the Waring blender. If there is an immediate need for re-use of the mixing container, the entire mixed sample may, at this point, be transferred to a suitable tumbler.

Allow the sample to stand for a period of at least 5 min but not to exceed 15 min.

Mix the sample thoroughly with a spoon for 5 sec and immediately fill the conical centrifuge tube to the 50 ml mark with liquid.

Centrifuge the tube 5 min at the required rpm.

Immediately siphon off the supernatant liquid to within 5 ml of the surface of the sediment level, using care not to disturb the sediment layer.

Add about 25 ml distilled water at a temperature of 75˚F and shake the tube gently to disperse the sediment, dislodging it, is necessary, with a wire.

Fill the tube to the 50 ml mark with distilled water at a temperature of 75˚F. Invert several times to mix the contents thoroughly.

Again centrifuge at the required rpm for 5 min.

Hold the tube in a vertical position with the upper level of the sediment on a level with the eye and report, to the nearest graduated scale division, the milliliters of sediment in the tube. The sediment is easily distinguished when the tube is held between the eye and a strong source of light.

Determination of Scorched Particles

Four procedures are in use for this determination. Spray-process samples and instant dry milk samples generally can be tested satisfactorily by the Water Disc Method, while roller-process samples require the Sodium Citrate or Calgon (sodium hexametaphosphate), or the EDTA Method.

Apparatus and Reagents:

A.D.P.I. Scorched Particle Standards for Dry Milks — photoprint published by the American Dairy Products Institute.*

Balance – Torsion, or similar type, approximately 500 g capacity and 0.1 g or better sensitivity.

Mixer – Waring Blender, or similar type.**

Scorched particle filtering discs – cotton discs 1 ¼" diameter, or cotton pads mounted on test cards for use with the aspirator-type tester. (Nelson-Jameson 800-826-8302, Visible Sediment Test Card Co. 515-795-3969 or Sediment Testing Supply Company 773-465-3634)

Scorched particle disc test cards – needed for individual discs only.

Scorched particle tester – aspirator, or pressure type, 1-1/8" filtering diameter.

Defoaming agent – Antifoam B emulsion, A 5757 (Nelson Jameson, Marshfield, WI or Ecolab, Inc., St. Paul, MN).

Water – sediment-free (distilled or efficiently filtered), preferably between 65-80ºF.

Sodium citrate – 10% solution. Dissolve 100 g of sodium citrate in distilled water and make up to 1 liter. Filter through a cotton disc before using.

Sodium hexametaphosphate (Calgon powder – unadjusted) – 2% solution. Dissolve 20 g of unadjusted Calgon in distilled water and make up to 1 liter. Filter cold through a cotton disc before using.

Tetra sodium salt of ethylenediamine tetra-acetic acid (EDTA) – 10% solution. Dissolve 100g of the chemical and make up to 1 liter. Filter cold through a cotton disc before use.

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* The intensity of color in the ADPI "Scorched Particle Standards for Dry Milks" photoprint may fade over a period of time, depending on exposure to light and the method of handling/storage. To minimize fading when not in use, the photoprint should be stored in a dark place (i.e., desk or file drawer), ideally between two sheets of black construction paper. Periodic replacement is recommended. If it is noted that a standard photoprint has faded, it should be replaced with a new standard.

**Malted milk-type mixers and the solubility index mixer do not always give comparable results. Waring Blender with worn or bent shaft should be replaced since metal particles may be produced, giving false scorched particle readings.

Determination of Scorched Particles (continued)

Procedure:

Water Disc Method – Spray-process Dry Milk Products

Measure 250 ml sediment-free water in Waring Blender jar. Start the mixer and add 25 g of nonfat dry milk, dry buttermilk, dry buttermilk product, or 32.5 g dry whole milk. Add approximately 0.5 ml of Antifoam B emulsion, A5757 and mix for 60 sec in the blender. Filter the entire solution through a standard cotton disc, using an aspirator or pressure-type tester.

Rinse the mixing container and tester with approximately 50 ml of sediment-free water, also passing this through the cotton disc.

If reliquefied sample is allowed to stand before filtering, stir vigorously just before pouring it into the tester. Do not allow samples to stand uncovered.

Remove the filter disc, place it in a scorched particle disc test card and dry at 30-40ºC in a dust-free atmosphere.

Compare the dry disc, placed on a table and viewed directly above with the A.D.P.I. Scorched Particle Photoprint under uniform, indirect light. Any test falling between two standard discs should be assigned the higher disc's letter. (Example: a disc showing more scorched participles than standard disc A but less than B should be assigned a B, and similarly for the other discs.)

Calgon Method – Roller-process Dry Whole Milk and Nonfat Dry Milk

Measure 250 ml of the 2% Calgon solution, heated to 80°F, into a Waring Blender jar. Turn on the mixer and add 17 g of well-mixed sample of nonfat dry milk or 22 g dry whole milk. Add approximately 0.5 ml of Antifoam B emulsion, A5757 and mix for 30 sec in the blender. Filter immediately through a standard cotton disc, using an aspirator or pressure-type tester.

Rinse the mixing container and tester with approximately 25 ml of sediment-free water, also passing this through the cotton disc. Place the disc in a scorched particle disc test card and dry at 30-40°C in a dust-free atmosphere.

Compare the dry disc, placed on a table and viewed from directly above, with the A.D.P.I. Scorched Particle Standards photoprint under uniform, indirect light. Any test falling between two standard discs should be assigned the higher disc's letter.

Determination of Scorched Particles (continued)

Sodium Citrate Method – Roller-process Dry Whole Milk and Nonfat Dry Milk

Measure 200 ml of hot (80-90°F) 10% sodium citrate solution into a Waring Blender jar. Turn on a mixer and add 17 g of well-mixed sample of nonfat dry milk or 22 g dry whole milk. Add approximately 0.5 ml of Antifoam B emulsion, A5757 and mix for 30 sec in the blender. Filter the entire solution through a standard cotton disc, using an aspirator or pressure-type tester.

Rinse the mixing container and tester with hot sediment-free water, also passing this through the cotton disc. Place the filter disc in a scorched particle disc test card and dry at 30-40ºC in a dust-free atmosphere.

Compare the dry disc, placed on a table and viewed from directly above, with the A.D.P.I. Scorched Particle Standards photoprint under uniform, indirect light. Any test falling between two standard discs should be assigned the higher disc's letter.

EDTA Method - Roller-process Dry Buttermilk and Dry Buttermilk Product

Place approximately 300 ml of hot (80-85°F) 10% solution of tetra sodium salt of ethylenediamine tetra acetic acid (dispersing agent) in the mixing jar of the high speed mixer. Turn on the mixer and add 17 g of dry buttermilk. Add a few drops of Antifoam B emulsion, A5757. Mix 8-10 sec. Add additional hot solution of the dispersing agent to the mixer until the volume of sample solution is approximately 500 ml. Agitate the sample for an additional 45 sec and filter immediately through a 1¼″ cotton disc. Rinse mixing container with hot water, passing all rinsings through the filter disc.

Compare the dry disc, placed on a table and viewed from directly above, with the A.D.P.I. Scorched Particle Standards photoprint under uniform, indirect light. Any test falling between two standard discs should be assigned the higher disc's letter.

Determination of Titratable Acidity

Apparatus and Reagents:

Balance – Torsion, or similar type, approximately 500 g capacity and 0.1 g or better sensitivity.

Burette – graduated in 0.1 ml divisions (for dispensing 0.1 N NaOH).

Casserole – white, glazed porcelain, 50 ml capacity (approximately 70 mm top diameter, 30 mm inside depth).

Indicator, phenolphthalein – 1.0% (prepared by dissolving 1.0 g phenolphthalein in 50 ml of 95% ethyl alcohol and diluting to 100 ml with distilled water).

Mixer – electric.

Pipette – volumetric, 17.6 ml.

Sodium hydroxide solution – 0.1 N.

Stirring rod – solid glass or Nafis type.

Water – recently boiled distilled.

Complete milk acidity testing outfits may be purchased as a unit; however, individual parts should conform to the specifications above.

Procedure:

Dissolve and disperse 10 g of nonfat dry milk, dry buttermilk, or dry buttermilk product, or 13 g of dry whole milk in 100 ml distilled water by using the mixer.

Allow the sample to stand for approximately 1 hr, stir gently and then pipette 17.6 ml into the porcelain casserole.

Rinse out the same pipette with 17.6 ml distilled water and add this to the sample in the casserole.

Add 0.5 ml phenolphthalein indicator and titrate with standardized 0.1 N NaOH until a faint pink color exists for 30 sec.

The number of ml 0.1 N NaOH required for the titration, divide by 20, gives the percentage titratable acidity (in terms of lactic acid) of the reliquefied sample.

Reference:

Standard Methods for the Examination of Dairy Products, current edition.

Determination of Dispersibility of Instant Nonfat Dry Milk (Modified Moats-Dabbah Method)

Apparatus and Regents:

Balance – accurate to ± 0.1 g.

Hydrometer – precision, specific gravity, range 1.0000-1.070, subdivisions 0.0005, Fisher 11-555 or equivalent.

Sieves – Tyler, 8", 60-mesh and 100-mesh, with pan.

Funnel – 150 mm.

Graduated cylinder – 250 ml.

Breakers – 600 ml.

Teaspoon

Thermometer - Centigrade, accurate to ± 0.5°C.

Defoaming agent – Antifoam B emulsion, A5757 (Sigma Chemical Co., St. Louis, MO).

Procedure:

Place 235 ml of distilled water tempered to 25°C ± 1°C, into a 600 ml beaker. Rapidly add 26 g of the dry milk sample and begin stirring immediately. Stir with an ordinary teaspoon by moving the spoon briskly back and forth across the diameter of the beaker 25 times in 15 sec, counting each time the spoon returns to its starting position.

Pour the mixture uniformly over the surface of the pre-wetted 60- and 100-mesh sieves mounted on a sieve pan. Allow a draining time of 1 min during which the sieves and pan are lightly rotated 3 times at 15-sec intervals. Separate and immediately tilt the sieves over the pan for an additional 30 sec to assist in complete drainage. Total drain time, 1 min 30 sec.

Add three drops of defoaming agent to the milk which has passed through the sieves. Pour the milk into a 250 ml granulated cylinder allowing the pan to drain completely. Add water to a volume of 250 ml. Mix by pouring the milk into a beaker and back into the graduated cylinder. Repeat this mixing step.

Place the thermometer and hydrometer into the milk. Allow to stand for 3 min. If foam is present, draw it off using a pipette attached to an aspirator. Record the temperature of the milk to the nearest degree Centigrade, remove the thermometer and take the hydrometer reading *to the first mark visible above the milk surface.*

Read the percent dispersion from the following table.

Determination of Dispersibility of Instant Nonfat Dry Milk(continued)

PERCENT MILK DISPERSED

Corrected Hydrometer Reading (H)	Temperature of Reconstituted Milk (C)		
	24	25	26
1.0200	61.1	61.9	62.4
1.0205	62.4	63.2	63.7
1.0210	63.7	64.5	65.0
1.0215	65.0	65.8	66.3
1.0220	66.3	67.0	67.7
1.0225	67.7	68.4	69.0
1.0230	69.0	69.8	70.3
1.0235	70.3	71.1	71.6
1.0240	71.6	72.4	72.9
1.0245	72.9	73.7	74.2
1.0250	74.2	75.0	75.6
1.0255	75.6	76.4	76.9
1.0260	76.9	77.7	78.2
1.0265	78.2	79.0	79.5
1.0270	79.5	80.3	80.9
1.0275	80.9	81.7	82.2
1.0280	82.2	83.0	83.5
1.0285	83.5	84.3	84.9
1.0290	84.9	85.7	86.2
1.0295	86.2	87.0	87.5
1.0300	87.5	88.3	88.9
1.0305	88.9	89.7	90.2
1.0310	90.2	91.0	91.5
1.0315	91.5	92.3	92.9
1.0320	92.9	93.7	94.2
1.0325	94.2	95.0	95.5
1.0330	95.5	96.3	96.9
1.0335	96.9	97.7	98.2
1.0340	98.2	99.0	99.6
1.0345	99.6	100.4	100.9

Notes:

1. *The hydrometer may be checked by completely dissolving 26 g ± 0.05 g of nonfat dry milk in 235 ml ± 0.1 ml of water and noting the temperature and hydrometer reading. This should correspond approximately to a 100% dispersed reading in the Table. If it does not, add or subtract an appropriate correction in use.*

Determination of Dispersibility of Instant Nonfat Dry Milk (continued)

Example: *Actual hydrometer reading at 26°C = 1.0370*
Hydrometer reading for 100%
Dispersibility at 26°C = 1.0340
Correction factor = 1.0370 – 1.0340 = 0.0030

The correction factor, once established, must be subtracted from each actual reading to get corrected hydrometer reading for obtaining results from table.

2. *A wet sieve is one which, after having been thoroughly rinsed or washed, has been lightly wiped with a towel to remove all excess water. The sieve pan is completely dried with a towel.*

3. *In reporting, round all results that are 0.5 or above to the next higher whole number.*

Examples: *74.2 report as 74%*
79.5 report as 80%
93.7 report as 94%

Determination of Flavor and Odor

Introduction:

Samples should be stored in a dark place or opaque container.

Apparatus and Reagents:

Balance – Torsion, or similar type.

Mixer - electric, or other type, and mixing container.

Water – distilled.

Procedure:

Immediately after opening the container, examine the sample of dry milk for flavor and odor.

The flavor and odor should be determined on a sample reconstituted as follows:

Add 10 g nonfat dry milk or dry buttermilk or 13 g dry whole milk to 100 ml distilled water and mix thoroughly.

Permit sample to stand 1 hr in a glass container with an air tight inert cap; gently stir and determine flavor and odor.

Determination should be made with sample at 75°F.

Report sample flavor as “good”, “fair” or “poor”.

Where possible, flavor characteristics should be identified.

Determination of Total Ash and Alkalinity of Ash

Apparatus and Reagents:

Balance – analytical.

Beaker - 400 ml, glass.

Bunsen burner

Burette – 50 ml in 0.1 ml graduations.

Calcium chloride – 40%; 40 g $CaCl_2$ dissolved in H_2O and diluted to 100 ml.

Crucible – platinum, 50 ml.

Furnace – muffle.

Hot plate

Hydrochloric acid – 0.1N; 86 ml of 36.5-38% HCl diluted to 10 L (AOAC current edition (936.15)).

Indicator, phenolphthalein – 1.0% (prepared by dissolving 1.0 g phenolphthalein in 50 ml of 95% ethyl alcohol and diluting to 100 ml with distilled water).

Sodium hydroxide – 0.1N; 54 ml of NaOH solution (1 + 1) diluted to 10 L (AOAC current edition (936.16)).

Steam bath

Stirring rod

Watch glass

Water – recently boiled distilled.

Procedure:

Total Ash

Carefully carbonize 2 g of dry milk in a 50-100 ml platinum crucible (5-6 cm bottom diameter) over a direct flame. Place the crucible and contents in a muffle furnace at 550°C for 1 hr. Cool; add a few milliliters of water to the ash and break up lumps with a flat-end stirring rod or policeman. Evaporate to dryness on a steam bath; re-ash in the muffle furnace for 1 hr and weigh the crucible and contents.

Determination of Total Ash and Alkalinity of Ash (continued)

Alkalinity of Ash

To the ash obtained above, add a few milliliters of water, break up and transfer to a 400 ml beaker with 50-75 ml of distilled water. Add 50 ml of 0.1 N H Cl; cover with a watch glass and transfer to a 400 ml beaker with 50-75 ml of distilled water. Add 50 ml of 0.1 N HCl; cover with a watch glass and heat to boiling. Boil gently for 5 min; cool and wash out the watch glass with recently boiled distilled water. Add 30 ml of 40% $CaCl_2$ solution (neutralized with 0.1 N H Cl and filtered) and let stand for 10 min.

Add approximately 10 drops of phenolphthalein indicator and titrate the excess acid with 0.1 N NaOH. The acid used (ml) X 50 equals the alkalinity of ash Express results as milliliters 0.1 N H Cl per 100 g of dry milk.

(All operations in this procedure must be carefully standardized.)

Reference:

Standard Methods for the Examination of Dairy Products, current edition.

Determination of Undenatured Whey Protein Nitrogen in Nonfat Dry Milk*

Apparatus and Reagents:

Test tubes – 25 x 150 mm lipless Pyrex or Kimax, or soft glass.

Funnels – short stem, diameter 50 mm, stem length 65 mm; short stem, diameter 90 mm, stem length 65 mm.

Filter paper – S & S No. 602, 9 cm; S & S No. 605, 15 cm pleated filter paper.

Cuvettes – selected round-cuvettes, 19 x 150mm (Coleman Instruments, Inc.)

Pipettes – Ostwald-Folin type, 1-, 2-, 3-, 4- and 5-ml; Volumetric, 5-, 10-, 20-, and 100-ml.

Balance – use an analytical balance for weighing the 2 g sample of NDM. Use a Torsion balance (± 0.1 g) to weigh the salt and the 20 g samples of NDM.

Water bath – thermostatically controlled to 37°C (± 1°C).

Spectrophotometer or colorimeter - the spectrophotometer or colorimeter being used by the individual laboratory.

Saturated sodium chloride – add one kilogram of cheese or butter salt to 2 liters of distilled water and heat the mixture to near boiling along with frequent stirring to insure complete saturation. After cooling to room temperature, filter the solution through S & S No. 605 pleated filter paper. Do not use Na Cl containing anti-caking agents for the preparation of this reagent or for saturation of the sample.

H Cl solution (10 g/100 ml) – 23 ml conc. H Cl, c.p. reagent plus 77 ml of distilled water.

Standard reference samples – low-heat NDM and high-heat NDM (Available from American Dairy Products Institute).

Procedure:

Standard Reference NDM samples need to be run frequently to match the curve. If discrepancies exist, check voltage, light bulb and cuvettes.

Reconstitute 2 g of NDM, in 20 ml of distilled water in a 25 x 150 mm test tube. Add 8 g of Na Cl, stopper and place in a water bath at 37°C for 30 min. Shake the contents of the tube from 8-10 times during the first 15 min of the incubation period, to insure complete saturation of the sample with Na Cl.

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* A modification of Harland-Ashworth method, published by Kuramoto, Jenness, Coulter and Choi. *Journal of Dairy Science. 42:28. 1959.*

Determination of Undenatured Whey Protein Nitrogen in Nonfat Dry Milk (continued)

Without cooling, shake mixture to facilitate pouring, and filter through S & S No. 602, 9 cm filter paper. Refilter through the same filter paper if the first portion of the filtrate is cloudy. Collect approximately 5 ml of filtrate.

Pipette a 1-ml aliquot of the filtrate into a cuvette. Dilute the filtrate with 10 ml of saturated Na Cl solution. Stopper the cuvette with a rubber stopper and mix by slowly inverting once.

Add 2 drops (delivered from a 5 ml volumetric pipette) of the H Cl solution, to develop the turbidity. Stopper the cuvette and mix the acid with the diluted filtrate by slowly inverting twice. Care should be taken to prevent the formation of foam.

At 5 minutes after adding the acid, invert cuvette once again and measure the turbidity in the instrument used in the particular laboratory, with the wave-length set at 420 nm. Adjust the instruments to 100% transmittance with a diluted casein-free filtrate made by diluting 1 ml of the original filtrate with 10 ml of saturated Na Cl solution.

Make duplicate determinations on each filtrate and, for each transmittance reading, obtain a value for serum protein nitrogen from the standard curve. Take an average of the duplicates and report as milligrams serum protein nitrogen per gram NDM.

Duplicates should agree within 2% transmittance. If they do not, another pair should be analyzed and the average of the four determinations used for the final value.

Preparation of Standard Curve:

Reconstitute 20 g (± 0.1 g) each of the standard low-heat NDM and the standard high-heat NDM with exactly 200 ml of distilled water in 500 ml Erlenmeyer flasks.

Saturate each reconstituted milk with 80 g of Na Cl, stopper the flasks, shake for 1 min and incubate at 37°C for 30 min. Shake the mixtures from 8 to 10 times during the first 15 min to insure complete saturation, allowing them to remain undisturbed for the remainder of the incubation period.

Without cooling or further agitation, except that necessary to permit pouring, filter the mixture through S & S No. 605 pleated filter paper. In cases where the first portion of the filtrate comes through cloudy, refilter through the same filter. Continue filtration until approximately 100 ml of filtrate have been collected. Cover the filters with a watchglass during the filtration, to prevent excessive evaporation.

Determination of Undenatured Whey Protein Nitrogen in Nonfat Dry Milk (continued)

Pipette proportions of low-heat and high-heat filtrates into 25 x 150 mm test tubes as follows:

Tube No.	Low-Heat Filtrate (ml)	High-Heat Filtrate (ml)
1	10	0
2	8	2
3	6	4
4	4	6
5	2	8
6	0	10

Stopper the tubes containing the combined filtrates and mix by slowly inverting the tubes twice.

Pipette 1 ml aliquots of the mixed filtrates into cuvettes, dilute with 10 ml of saturated Na Cl, and develop the turbidity as described previously.

Plot the standard curve, using serum protein nitrogen values (Kjeldahl) per gram of sample for the horizontal axis and per cent transmittance as the vertical axis on 8½ x 11″ graph paper (1 mg SPN equals 1″, 10% transmittance equals 1″). It is essential that the difference in transmittance readings between the low-heat and high-heat filtrates be as large as possible, preferably at least 35%.

Day-to-day reproducibility of the instrument should be checked following recommendations of the manufacturer.

NOTE:

Whey Protein Nitrogen (WPN) values obtained by a modified Kjeldahl analysis on standard reference samples are corrected to a 3.16% moisture content.

To correct the WPN value of nonfat dry milk to a 3.16% moisture content, use the following equation:

$$\text{WPN @ 3.16\% } H_2O = \frac{[100 + (\%\ H_2O - 3.16\%)]\ [\text{WPN value}]}{100}$$

Determination of Powder Bulk Density

Definition:

Powder Bulk Density = g/ccm as determined under the conditions described below.

Apparatus and Reagents:

Balance – sensitivity 0.1 g.

Brass Cylinder – with detachable top. The volume of the lower cylinder is exactly 100 ccm.

Stampf-volumeter – made by Engelsmann, Ludwigshafen, Germany.

Procedure:

Weigh the cylinder without the top.

Put the top on the cylinder and fill up to the rim with powder using a spoon.

Remove the top and scrape off powder until it is flush with the rim of the cylinder.

The weight of the powder indicates loose bulk density.

Repeat procedure and tap the cylinder 10 times.

Weigh this and it represents the packed powder bulk density.

Repeat procedure and tap further 90 times, scrape off and weigh. The weight of the powder indicates final powder bulk density.

Calculation:

Weight of the powder/100 = powder bulk density

Reproducibility:

+/- 0.03 g/ccm for poured bulk density

+/- 0.01 g/ccm for tapped 100 and 1250 times.

Other Approved Methods of Analysis

Fat – AOAC 989.05; ISO1736/IDF 9C

Total Moisture – AOAC 925.45; AOAC 927.05 (Vacuum Oven); ISO 5537/IDF 26

Protein – AOAC 991.20 (N x 6.38); ISO 8968-1/IDF 20 part 1

Ash – AOAC 900.02; AOAC 942.05

Lactose (anhydrous) – ISO 22662/IDF 198