

Nonprotein Nitrogen Determination for Skim Milk Powder and Nonfat Dry Milk

Principle

Biologically derived protein-based food ingredients inherently contain nonprotein nitrogen (NPN) compounds the quantity of which can be a useful diagnostic tool to help substantiate the identity of the ingredient. The amount of NPN naturally occurring in skim milk powder and nonfat dry milk falls within a narrow range around a mean of ~0.3% and is composed of urea, creatine, and other nitrogen-containing compounds. If certain intentional adulteration(s) are carried out at a significant level, the addition of nitrogen-containing chemical adulterants, such as melamine or urea, to skim milk powder or nonfat dry milk to increase the apparent protein level as measured by many traditional test methods, can increase the measured total NPN content of the ingredient above the normal range and result in a material failing to meet acceptance criteria. Similarly, sufficient dilution of skim milk powder or nonfat dry milk with nonmilk-based fillers may result in a decrease in NPN content that would result in a material failing to meet the acceptance criteria.

Two methods for determining NPN content are available, each measuring the total nitrogen content (using Kjeldahl nitrogen methodology) of the nonprotein fraction isolated from the sample. The NPN fraction is isolated using either precipitation of the protein away from NPN by tannic acids, or by direct extraction of NPN using molecular size filtration. The measured NPN content is compared to expected NPN range boundaries specific for that method for authentic skim milk powders and nonfat dry milks. These boundaries were calculated statistically at a 95% confidence level taking into account sample and method variance. Samples failing to meet the acceptance criteria in this standard are suspected to be adulterated, and thus require additional evidential analyses by other qualitative and quantitative methods for confirmation.

The estimated detection capabilities of both methods for seven model, nitrogen-rich adulterants have been studied in single laboratories. The results are presented in *Table 7*. These results indicate the minimum level (% w/w basis) of adulterant, that when added to a sample, increases the NPN content above the 95% confidence level for nonadulterated materials.

Table 7. Estimated Detection Capabilities^a

Adulterant	Molecular Size Filtration	Tannic Acid Precipitation
Melamine	0.06%	0.07%
Urea	0.09%	0.04%
Ammonium phosphate	0.22%	0.05%
Isobutylidene diurea (IBDU)	0.16%	0.09%
Aminotriazole	0.11%	0.05%

^a The minimum level (% w/w basis) of adulterant, that when added to a sample, increases the NPN content above the 95% confidence level for nonadulterated materials.

Table 7. Estimated Detection Capabilities³ (continued)

Adulterant	Molecular Size Filtration	Tannic Acid Precipitation
Dicyandiamide	0.07%	0.05%
L-Arginine	0.15%	0.06%

³ The minimum level (% w/w basis) of adulterant, that when added to a sample, increases the NPN content above the 95% confidence level for nonadulterated materials.

Scope: This standard is applicable to skim milk powders and nonfat dry milks.

Change to read:

• METHOD 1: MOLECULAR SIZE FILTRATION

Sample solution: 10.0% (w/w) prepared by combining a sample and water in a suitable container and blending contents with a homogenizer⁷ at 14,000 rpm for 20 s.

Analysis: Fill to capacity with *Sample solution* a centrifugal filter device with a 3K nominal molecular weight limit.⁸ Centrifuge at the maximum speed recommended by the centrifugal filter manufacturer until at least 2.0 mL of filtrate can be collected. [NOTE—Centrifugation up to 1 h may be necessary depending on exact conditions.] Analyze an aliquot of filtrate (NLT 2.0 mL) for total nitrogen content (TN) using the procedure described in Nitrogen Determination, Appendix IIIC.

Calculate the %NPN in the portion of the sample taken:

$$\text{Result} = (\text{TN}/C_U) \times 100$$

TN = total nitrogen content of the aliquot analyzed (% w/w, expressed as a decimal)

C_U = concentration of the *Sample solution* (% w/w, expressed as a decimal)

System suitability

Sample: USP Skim Milk Powder RS • (IS 1-Jun-2016)

Suitability requirement: The relative standard deviation for the NPN content of USP Skim Milk Powder RS is NMT 7.5% for 3 replicate analyses. • (IS 1-Jun-2016)

Acceptance criteria: 0.242%–0.322% [NOTE—Materials failing to meet this criteria are suspected of adulteration and should be further investigated with confirmatory methods.]

• METHOD 2: TANNIC ACID PRECIPITATION

12% (w/w) Tannic acid solution: Dissolve 12 g of tannic acid⁹ in 88 g of water in a 4-oz screw cap bottle. Cap, and shake until dissolution is complete. [NOTE—Solution may darken, but is stable indefinitely.]

⁷ Ultra Turrax T 18 with a S18N-10 g dispersion tool (available from IKA, Wilmington, NC, catalog numbers 0003720001 and 003452400, respectively), or equivalent.

⁸ Amicon Ultra-15 with Ultracel-3K membrane (available from Millipore, catalog # UFC800396), Ultra 4 Centrifugal Filter Unit with Ultracel-3K membrane (available from Millipore, catalog # UFC900324), or equivalent.

⁹ ACS reagent grade (Sigma Aldrich catalogue number 403040), or equivalent.

1% (w/w) Sodium chloride solution: Dissolve 2 g of sodium chloride in 198 g of water in an 8-oz screw cap bottle. Cap, and shake until dissolution is complete.

Sample solution: Weigh 200.0 mg of the sample into a 15-mL centrifuge tube, and record the sample weight (M_0) to the nearest 0.1 mg. Add 6.00 g of 1% Sodium chloride solution. Mix thoroughly with a vortex mixer, and allow to stand for at least 15 min. Mix briefly on a vortex mixer to assure homogeneity. Add 3.00 g of 12% Tannic acid solution, and record the combined weights of added 1% Sodium chloride solution and 12% Tannic acid solution, (M_1) to the nearest 0.1 mg. Mix thoroughly with a vortex mixer, and allow to stand for at least 30 min. Centrifuge the precipitate from solution for 15 min at $1000 \times g$. [NOTE—A swinging bucket centrifuge is most convenient for subsequent removal of the supernatant.] Carefully transfer most of the supernatant (~6 mL) to a suitable container, and use it in its entirety as the *Sample solution* for Nitrogen Determination, Appendix IIIC.

Analysis: Determine the TN of the *Sample solution* using the procedure described in Nitrogen Determination, Appendix IIIC.

Calculate the %NPN in the portion of the sample taken:

$$\text{Result} = \text{TN} \times [(M_0 + M_1)/M_0]$$

TN = total nitrogen content of the *Sample solution* (% w/w)

M_0 = weight of sample used to prepare the *Sample solution* (g)

M_1 = combined weight of 1% Sodium chloride solution and 12% Tannic acid solution used to prepare the *Sample solution* (g)

System suitability

Samples: USP Skim Milk Powder RS and USP Skim Milk Powder with Melamine–Level D RS

Suitability requirements

Suitability requirement 1: The relative standard deviation for the NPN content of USP Skim Milk Powder RS is NMT 6.0% for 3 replicate analyses.

Suitability requirement 2: The measured %NPN content for USP Skim Milk Powder with Melamine–Level D RS is NLT 0.054 greater than the measured content for USP Skim Milk Powder RS.

Acceptance criteria: 0.251%–0.337% [NOTE—Materials failing to meet this criteria are suspected of adulteration and should be further investigated with confirmatory methods.]