

A 21 a - Whey Protein Nitrogen Index

GEA NIRO® Method No. A 21 a

Revised: January 2024

1. Principle

The undenaturated Whey Protein Nitrogen Index (WPN) is a measure of the heat treatment applied to the milk during processing to milk powder. It is the basis of the following heat classifications:

Class	WPN
High heat powder	< 1.5
Medium heat powder	> 1.5 - < 6.0
Low heat powder	> 6.0

WPN is expressed as milligrams (mg) undenaturated whey protein nitrogen per gram of non-fat milk powder with a moisture content of 3.16%.

Casein and the heat denaturated whey proteins are removed by filtration after precipitation of the reconstituted milk with NaCl. The filtrate contains all undenaturated whey proteins. By adding HCl, the proteins denature and develop a turbidity depending on the concentration of the whey proteins. The turbidity is measured as % transmittance, in a spectrophotometer at a wave length of 420 nm.

By using the standard curve, this reading can be converted directly into mg undenaturated whey protein N/g powder (WPN).

2. Scope

The method is to be used for milk powders, liquid milk and milk concentrates.

3. Apparatus

1. Spectrophotometer - Perkin Elmer, Lambda 2, UV / VIS.
 2. Analytical balance - capable of weighing to 0.01 g.
 3. Water bath - thermostatically controlled to $37 \pm 0.5^\circ$ C.
 4. Electrical heat plate.
 5. Vibrator - Vibrofix VF 1, Electronic.
 6. Test tube - 25 x 150 mm round bottom, Pyrex or Kimax, or soft glass. Rubber stoppers.
 7. Glass funnel - short stem 65 mm, diameter 50 mm. Glass funnel - short stem 65 mm, diameter 90 mm.

8. 15 ml specimen tube with stopper - socket size 14/23.
9. Pleated filter paper - 9 cm, S & S No. 602. Pleated filter paper - 15 cm, S & S No. 605.
10. Volumetric pipettes - 1 - 2 - 3 - 4 - 5 - 10 - 20 - 100 ml. Disposable pipette.
11. 100 ml volumetric flask.
12. 50 ml + 500 ml Erlenmeyer flasks.
13. 2000 ml graduated beaker.
14. 2 l bottle with a lid.
15. Stop watch.
16. 60 mm + 90 mm watch glasses.
17. Weighing dish, disposable.

4. Chemicals

1. Hydrochloric acid, fuming, p.a. Merck.
Conc. HCl 37%. R 34-37 and S 2-26.
2. Sodium chloride (NaCl), p.a., Merck.
Do not use NaCl containing anti-caking agents.
3. Standard reference samples.
Low heat and high heat non-fat dry milk (NDM).
(Available from the American Dairy Products Institute)

R ≈ DK risk sentences

S ≈ DK safety sentences

5 Reagents

1. HCl solution (10 g/100 ml).
Add 23.0 ± 0.1 ml conc. HCl to 77 ml deionized water.
2. NaCl, saturated.
Add 1 kg of NaCl to 2 l of deionized water and heat the mixture to just below boiling point while stirring to ensure complete saturation. After cooling to room temperature, filter the solution through S & S No. 605 pleated filter paper.

6. Standard Curve

1. Reconstitute 20 ± 0.01 g of both standards with 200.0 ml of deionized water in 500 ml Erlenmeyer flasks.
2. Add 80 g of NaCl to saturate the reconstituted milk. Stopper the Erlenmeyer flask, shake for 1 min., and incubate for 30 min. at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ in a water bath. Turn the solution upside down 8-10 times during the first 15 min. to insure complete saturation. Do not touch the solution in the last 15 min. of the incubation period.
3. Without cooling, filter the solution through S & S No. 605 pleated filter paper. If the first portion of the filtrate is cloudy, re-filter through the same filter paper. Continue filtration until approx. 100 ml of filtrate have been collected. Cover the funnel with a watch glass during filtration, to prevent evaporation.
4. Pipette proportions of low heat and high heat filtrates into 25 x 150 mm 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 with the combined filtrates, and mix carefully by inverting twice. Continue as described in 7.5 - 7.10

5. Make duplicate determinations of each point.
6. Check that each measurement is within the confidence limit of the existing standard curve. If that is the case continue to use the curve, if not, calculate the linear regression curve that fits the numbers measured with the new standard samples. Stat Graphic program or equivalent is suitable. An example of a standard curve is shown in Fig. 1 and Fig. 2.
7. New standard samples have to be bought every 6 months and used for control of the standard curve.

7. Procedure

1. The method uses 2.0 g of non-fat dry powder.
For milk powders containing more than 1% fat, a correction in the amount of sample used for analysis is required.

$$\text{Amount of powder for analysis} = \frac{20 \times 100}{(100 - \% \text{fat})}$$

% fat can be determined by using Gerber Method, GEA Niro Method A 9 b

2. Reconstitute the milk powder in 20.0 ml of deionized water, in a 25 x 150 mm test tube. Make duplicates from each sample. Use the Vibrofix until all milk powder is dissolved.
3. Add 8.0 ± 0.1 g of NaCl, stopper, and place the test tube in a water bath at $37 \pm 0.5^\circ\text{C}$ for 30 min. Shake the content of the tube 8-10 times during the first 15 min. to ensure complete saturation of the milk solution with NaCl.
4. Without cooling, shake the mixture to facilitate pouring, and filter through S & S No. 602 filter paper. If the first portion of the filtrate is cloudy, re-filter through the same filter paper. Collect approx. 5 ml of the filtrate in a 50 ml Erlenmeyer flask.
5. Pipette 2 x 1.0 ml of the filtrate, one for a sample and one for a blind determination, into two 15 ml tubes. Dilute the filtrates with 10 ml of saturated NaCl solution. Stopper

- the tube and mix by slowly inverting once.
6. Add 2 drops of HCl solution (use a disposable pipette) to the sample (not the blind sample) for developing a turbidity. Stopper the tube and mix the acid with the diluted filtrate by slowly inverting twice. Do it very carefully to prevent formation of foam.
 7. Adjust the spectrophotometer to 100% transmittance by using the blind sample, with the wavelength set at 420 nm.
 8. Rinse carefully with deionized water between each transmittance reading.
 9. Within 5-10 min. after adding the HCl solution, invert the tube once again and measure the turbidity on the spectrophotometer with the wavelength set at 420 nm.
 10. Before measuring the duplicate sample, check the 100% transmittance reading with the corresponding blind sample.

8. Result

1. Transmittance readings on the duplicates must not differ by more than 2%. If they do, another pair of filtrates must be analyzed, and the average of 4 determinations is used as the final value.
2. To obtain a value for serum protein nitrogen, use the Standard Curve. Report the result as: mg WPN/g powder NDM.
3. Whey Protein Nitrogen (WPN) values obtained by a modified Kjeldahl analysis on standard reference samples are corrected to a 3.16% moisture content. Therefore measured WPN values have to be corrected to the same water content.

$$WPN \text{ at } 3.16\% H_2O = \frac{(100 + (\%A - 3.16\%)) \times (WPN)}{100}$$

A = % moisture in the sample (GEA Niro Method A 1 a)

9. Remarks

1. When producing skimmed milk powder with a certain heat-treatment specification, it is necessary to know the whey protein content in the raw skim milk as well as the heat stability of the whey proteins. Both are influenced by seasonal variations. Therefore the heat treatments applied to the milk prior to evaporation must differ through the year in order to meet required specifications.
2. Liquid skimmed milk, skim milk concentrates, whole milk powder etc.:
The amount of sample and water for step 7.1 must be calculated in such a way to obtain approx. the same amount of non-fat solids and water in the sample as when dissolving 2.0 g of skim milk in 20 ml of water. The result found is expressed in mg WPN/1 g of skim milk powder and can be used directly for heat classification.
3. Whey powder:
Use 1.0 g of whey powder and 20 ml of water for step 7.1 and multiply the result of step 8.3 by 2. The result is expressed as mg WPN/1 g powder. In the case of whey powder express the degree of denaturation in %. Multiply the result by 0.638 to obtain the amount of undenaturated whey protein of the powder in %. Simultaneously determine the total protein content by using the Kjeldahl method, and calculate the % of denaturation.
4. It is recommended not to filtrate the milk for more than approx. 2 hours, as there is a risk of crystallization in the residue.

Fig. 2. Calculation of Standard Curve

Regression Analysis - Linear model: $f(x) = a+bx$

 Dependent variable: WPN. WPN Independent variable: WPN. average

Parameter	Estimate	Std. error	T-value	Probe level
Intercept	16.8753	0.0765988	220.308	0.00000
Slope	-0.178078	1.0303 SE-3	-172.827	0.00000

 __ Analysis of variance

Source	Sum of squares	Df	Mean square	F-Ratio	Probe level
Model	32.011	1	32.011	29869.26	0.00000
Residual	0.0042868	4	0.0010717		
Total (corr.)	32.015400	5			

 __ Correlation coefficient = - 0.999933

R-squared = 99.99%

Standard error of estimate = 0.0327369