DEPARTMENT OF THE TREASURY
ALCOHOL AND TOBACCO TAX AND TRADE BUREAU
SCIENTIFIC SERVICES DIVISION
WASHINGTON, DC 20220

OFFICIAL METHOD — SSD:TM:504

Protein in Wine

Scope and Application

This method is used to determine protein in standard wines, ciders, and sake. The objective of this method is to determine the nitrogen content as a basis for the calculation of the protein level. TTB Procedure 2004–1 requires that all Alcohol Facts Labels include a statement of average analysis for calories, fat, carbohydrate, and protein.

The principle of this method is based on the classical Kjeldahl method for the determination of protein. The sample is first digested in boiling sulfuric acid, which converts nitrogen to ammonium ion. A copper catalyst is used to promote the digestion process. After digestion is complete, the solution is made basic and is distilled using steam into a receiving flask containing a known amount of sulfuric acid. Excess, unreacted acid is then titrated with standard sodium hydroxide to determine how much was consumed by the distilled ammonia. Each mole of nitrogen in the protein produces one mole of ammonia. Protein contains 16% by weight nitrogen. Thus, by multiplying the nitrogen determined by 6.25, the protein content of the sample is obtained.

Regulatory Tolerances

The regulatory tolerance limits for protein labeling are listed in TTB Procedure 2004–1, which states:

> The statement of protein content on labels or in advertisements for alcohol beverages will be considered acceptable as long as the protein content, as determined by TTB analysis, is within a reasonable range above the labeled or advertised amount (within good manufacturing practice limitations) but must not be more than 20% below the labeled or advertised amount.

The protein content on the label must be expressed as follows:

> A statement of the number of grams of protein in a serving must be expressed to the nearest tenth of a gram, except that if a serving contains less than 1 gram, the statement “Contains less than 1 gram” or “less than 1 gram” may be used as an alternative, and if the serving contains less than 0.5 gram, the content may be expressed as zero.

Levels and Limitations

Analyte	*Detection Limit (ppm)*	*Quantitation Limit (ppm)*	*Linear Range (ppm)*	*Interferences*
Protein	44*	88**	Not Applicable	None

* LOD determined by calculation, assuming 0.05 mL of titrant and 10 mL of sample.

** LOQ determined by calculation, assuming 0.10 mL of titrant and 10 mL of sample.

Equipment

- Buchi Nitrogen Digestion Unit, Model 425 or equivalent.
- Buchi Distillation Unit 323 or equivalent.
- Man-Tech PC-Titrate System or equivalent.
- Steam bath.
- Volumetric glassware, Class A.
- Analytical balance, records to at least 1 mg.

Reagents, Sample Preparation and Handling

Reagents:

- DI water, Millipore Milli-Q grade or equivalent.
- Di-sodium ethylenediamine tetraacetate (Na_2EDTA), reagent grade, available from Fisher Scientific.
- Kjeldahl tablets CT-37 (3.5 grams K_2SO_4 and 0.1 grams $CuSO_4$ per tablet), available from Fisher Scientific.
- Sulfuric acid, concentrated, reagent grade, available from Fisher Scientific.
- Sodium hydroxide, aqueous solution 50% w/w, available from Fisher Scientific.
- Sulfuric acid, 0.1 N standard solution, available from Fisher Scientific.
- Sodium hydroxide, 0.1 N standard solution, available from Fisher Scientific.
- Ethanol, 200 proof, available from Gold Shield Distributors, Hayward, CA.

Reagent Preparation:

Nitrogen Standard Solution —

1. Na_2EDTA (FW 372.24) yields 0.0752 Nitrogen (g/g).
2. Using an analytical balance, weigh 6.0 grams of Na_2EDTA and record the weight to the nearest milligram.
3. Place in a 1 L volumetric flask. Q.S. with 5% v/v ethanol/water.
4. This concentration will yield about 0.4 g/serving for wine.
5. Store in refrigerator for up to 1 year or when the measured N has dropped 5% below the label value.

25% Aqueous NaOH —

1. This will generate considerable heat and fumes — please see Safety Notes below.
2. Place a sturdy, plastic vessel in a cooling ice bath.
3. Slowly and carefully add NaOH 50% w/w to an equal volume of water and mix well.
4. Allow the solution to cool to room temperature before proceeding.

Sample Preparation:

1. If sample is carbonated, de-gas according to SSD:WG:103.
2. Reagent blank uses 5 mL of DI water in lieu of sample.
3. Prepare a titrator check sample by pipetting 10.0 mL of 0.1 N sulfuric acid into a receiving (titration) beaker and then adding sufficient DI water to bring it to the 60 mL mark on the beaker.

Procedures

1. Pipette 10.0 mL of each sample into a Buchi digestion tube. Mark each tube with the sample number using indelible ink.
2. Pipette 5 mL of standard or water (for reagent blank) into Buchi digestion tubes. Mark these tubes as standard or reagent blank as appropriate.
3. Reduce to a syrup or to dryness on a steam bath.
4. Add a catalyst tablet and 10 mL conc. sulfuric acid.

5. Digest at 80% power using Buchi digestor connected to an aspirator, until thick, white fumes clear; digest 30 minutes more. Total digestion time is approximately 90 minutes. Cool to room temperature before proceeding.
6. Prepare receiving (titration) beakers for the distillation by pipetting 10.0 mL of 0.1 N sulfuric acid into each beaker. If needed, add up to 10 mL of DI water to each beaker to cover the tip of the distillation tube.
7. Place digestion tube on Buchi distillation apparatus; hydrolyze with 75 mL of water, followed by 75 mL of 25% aq. NaOH.
8. Distill into receiving beaker containing the 10.0 mL of 0.1 N sulfuric acid for 3 minutes, collecting about 60 mL total of solution.
9. Titrate the titration check sample first to check the titrator for accuracy.
10. Titrate distillate to pH 6.0 with 0.1 N NaOH.

Quality Control

1. Include a titrator check sample, a reagent blank, and a nitrogen standard with each daily run. The reagent blank should not differ from the titration check sample by more than 0.05 mL of titrant. The nitrogen standard should not differ from the calculated value by more then 5%.

2. Calibrate the pH mater of the autotitrator daily using the procedure supplied by the instrument manufacturer.

Calculations

$$\text{Protein g/serving} = \frac{[(V_a \times N_a) - (V_b \times N_b)] \times 14.007 \times 6.25 \times V_c}{1000 \times V_s}$$

Where: V_a = Volume of 0.1 N sulfuric acid.

N_a = Normality of 0.1 N sulfuric acid.

V_b = Volume of 0.1 N sodium hydroxide used in the titration.

N_b = Normality of 0.1 N sodium hydroxide.

V_c = Volume serving in mL; 5 fluid ounce serving = 148 mL.

V_s = Volume of sample, blank or standard pipetted.

Reporting Results

Report protein to one decimal place, i.e. XX.X g protein/5 fl.oz.

Safety Notes

- Normal laboratory safety protocol should be followed.
- Preparation of 25% Aqueous NaOH requires special personal protective gear and a chemical fume hood. This will generate considerable heat and fumes.

References

- TTB Procedure 2004–1.
- ATF Ruling 80–3.
- D. Harris, Quantitative Chemical Analysis, 5th Ed., W.H. Freeman & Co., New York, 1999, pp. 151-153.