27.1.25A

AOAC Official Method 997.09
Nitrogen in Beer, Wort, and Brewing Grains
Protein (Total) by Calculation

Combustion Method
First Action 1997

ASBC–AOAC Method

(Applicable to beer and wort containing 0.01–0.15% N and solid brewing grains containing 0.1–9% N.)

See Table **997.09** for the results of the interlaboratory study supporting the acceptance of the method.

A. Principle

Nitrogen is freed by combustion of test portion in pure O. Nitrogen gas is measured using thermal conductivity detector. Total N is calculated based on response from known N standard. Nitrogen is converted to protein by multiplying by 6.25.

B. Apparatus

(**a**) *Nitrogen combustion analyzer*.—With thermal conductivity detector, capable of reducing NO_x compounds to N. Any instrument that offers range of 0.02–100 mg N, precision of 0.002 or 0.75% RSD (whichever is greater), and readability of 0.001% (or better) for N determination is suitable.

(**b**) *Laboratory mill*.—With 0.5 mm or smaller screen.

(**c**) *Analytical balance*.—Weighing to nearest 0.1 mg.

C. Reagents

(**a**) *Nitrogen standard solution for liquid*.—Lysine hydrochloride reagent (15.33% N). Weigh ca 1 g lysine hydrochloride to nearest 0.1 mg and dissolve in 100 mL weighed amount of H_2O. Percent N (by weight) = [weight of nitrogen standard (g) × 15.33]/[weight of nitrogen standard (g) + weight of H_2O (g)] = ca 0.152.

(**b**) *Nitrogen standard for solid*.—Ethylenediamine tetraacetic acid (EDTA) 9.59% N.

(**c**) *Check standard for solid*.—Nicotinic acid *p*-toluene sulfonate (Hach Company, PO Box 389, Loveland, CO 80539, USA, Catalog # 22781-24), 4.743% N.

(**d**) *Compressed oxygen gas*.—99.99%.

(**e**) *Compressed helium gas*.—Nitrogen-free, 99.99%.

D. Determination of Nitrogen

Mill grain before analysis. No preparation is needed for liquids unless they exceed calibration range and require dilution. Use N-free H_2O for dilutions. Include dilution factor in calculation.

When operating instrument, follow instrument manufacturer's recommendations for size and introduction of test portion, furnace temperatures, burn times, flow rates, and other operational settings.

Establish instrument's baseline by running at least 3 reagent (carrier gas) blanks. Calibrate instrument for liquids by analyzing at least 3 replicates of N standard solution, **C**(**a**). Calibrate for grains by analyzing at least 3 replicates of N EDTA standard, **C**(**b**), and check midrange response using NTS, **C**(**c**). Calculate the average response factor.

E. Calculations

Calculate response factor (*RF*) as follows:

$$RF = \frac{\%N_{std} \times W_{std}}{P_{std} - P_b}$$

where % N_{std} = % N in standard, **C**(**a**) or **C**(**b**); W_{std} = weight of standard, g; P_{std} = area count of standard; and P_b = area count of blank.

Calculation of % N of test material is usually performed by integrator of instrument as follows:

$$\%\ \text{N, by weight} = \frac{(P_{test} - P_b) \times RF}{W_{test}}$$

where P_{test} = area count of test portion; P_b = area count of blank; *RF* = appropriate response factor; and W_{test} = test portion weight, g.

Report results for beer and wort to nearest 0.001% N and grain to nearest 0.01% N.

Calculate % protein in test portion as follows:

$$\text{Protein, \% by weight} = \%\ \text{N, (by weight)} \times 6.25$$

where 6.25 = nitrogen-to-protein conversion factor.

Report results to nearest 0.01% protein.

References: ASBC: Beer **11B**, Barley 7C.
J. AOAC Int. (future issue).

Table 997.09 Interlaboratory study results for determination of nitrogen in beer, wort, and brewing grains by combustion method

Sample	No. of labs	Mean N, %	s_r	s_R	RSD_r, %	RSD_R, %	r[a]	R[b]
Spent grain	12	4.019	0.219	0.270	5.5	6.7	0.614	0.756
Barley	12	2.160	0.036	0.091	1.7	4.2	0.102	0.193
Malt	12	1.526	0.033	0.069	2.2	4.5	0.093	0.193
Rice	10	1.072	0.015	0.024	1.4	2.2	0.041	0.066
Lab extract 1	10	0.0769	0.0018	0.0025	2.3	3.3	0.0050	0.0070
Lab extract 2	10	0.1179	0.0033	0.0053	2.8	4.5	0.0093	0.0148
Production wort 1	10	0.1345	0.0022	0.0058	1.6	4.3	0.0061	0.0162
Production wort 2	10	0.1028	0.0014	0.0043	1.4	4.1	0.0039	0.0119
Beer 1	9	0.0723	0.0012	0.0033	1.6	4.6	0.0033	0.0093
Beer 2	10	0.0340	0.0028	0.0028	8.3	8.2	0.0079	0.0078

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.