Total Nitrogen in Brewing Grains by Combustion Method

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CONCLUSIONS

1. The combustion method produced acceptable repeatability and reproducibility coefficients of variation for the analysis of brewing grains with 1–5% nitrogen.
2. The method was found to have a detection limit of 0.0321% N and a determination limit of 0.1115% N.

RECOMMENDATIONS

1. The method as given in the Appendix is recommended for inclusion in *Methods of Analysis* for brewing grains having 1–5% nitrogen (approximately 6–30% protein).
2. The subcommittee should be reassigned to examine the combustion method for liquid samples and for brewing materials with less than 1% nitrogen.
3. Ethylenediaminetetraacetic acid at 9.59% nitrogen should be used as the calibration standard.

This subcommittee was charged with evaluating the combustion method for the analysis of percent total nitrogen in brewing grains (2). This method provides for faster and safer analysis of total nitrogen than the current Kjeldahl method (2,4).

PROCEDURE

Four sample pairs consisting of brewing grains (rice, barley, malt, and spent grains) and a calibration standard (nicotinic acid *p*-toluene sulfonate, 4.743% N) were sent to each collaborator. The collaborators were asked to prepare the sample and analyze for percent total nitrogen using the combustion method. Samples were analyzed in duplicate and results reported. Average values were used to compare results. The Youden unit block (3) experimental design was used.

RESULTS AND DISCUSSION

Eleven collaborators submitted 12 sets of results from three types of instruments (Table I). All samples were checked for outliers using Dixon's outlier test (1), and no outliers were found. The statistical summary is shown in Table II. Repeatability and reproducibility coefficients of variation were acceptable for all sample pairs.

The limits of detection and determination (1) were measured in the chairman's laboratory by analyzing 10 reagent blanks (carrier gas). The results ranged from −0.02 to 0.02% N, with a mean of 0.0020% N and a standard deviation of 0.0108. Based on these results, a limit of detection of 0.0321% N and limit of determination of 0.1115% N were calculated at a precision coefficient of variation of 10%.

A calibration curve was developed by analysis of four organic compounds and reagent blanks having a nitrogen content ranging from 0 to 9.59%. Linear regression analysis of the results demonstrated excellent linearity.

TABLE I
Total Nitrogen in Brewing Grains by Combustion Method (%)

Collaborator	Sample Pair A	B	Sample Pair C	D	Sample Pair E	F	Sample Pair G	H
1	3.92	3.49	2.07	2.13	1.47	1.52	1.06	1.07
2	3.76	3.92	2.12	2.15	1.51	1.52	1.09	1.07
3	4.03	4.46	2.36	2.23	1.54	1.55	1.10	1.12
4	4.13	4.07	2.16	2.19	1.57	1.62	1.10	1.11
5	4.02	3.99	2.09	2.05	1.48	1.46	1.07	1.08
6	4.44	4.35	2.34	2.30	1.67	1.63	1.21	1.19
7	3.85	3.77	2.07	2.08	1.47	1.47	1.04	1.06
8	4.03	4.00	2.17	2.19	1.54	1.60	1.05	1.09
9	4.06	3.78	2.07	2.12	1.46	1.46	1.07	1.06
10	3.65	3.87	2.10	2.12	1.48	1.47	1.06	1.06
11	3.79	4.51	2.11	2.09	1.41	1.52	1.06	1.03
12	4.34	4.23	2.26	2.26	1.63	1.57	1.17	1.14
Mean	4.000	4.036	2.162	2.158	1.518	1.539	1.089	1.089
Grand mean	4.018		2.160		1.528		1.089	

TABLE II
Statistical Summary of Results[a]

Sample Pair	No. of Labs.	Grand Mean	Repeatability s_r	cv_r	r_{95}	Reproducibility s_R	cv_R	R_{95}
A/B	12	4.018	0.219	5.5	0.613	0.219	6.7	0.753
C/D	12	2.156	0.036	1.7	0.102	0.092	4.2	0.256
E/F	12	1.528	0.030	2.0	0.085	0.074	4.8	0.207
G/H	12	1.089	0.015	1.3	0.041	0.047	4.3	0.131

[a] All calculations were made based on reference 3.

Several collaborators expressed dissatisfaction with the standard that they were supplied, nicotinic acid *p*-toluene sulfonate; therefore it is recommended that ethylenediaminetetraacetic acid (9.59% N) be used as the calibration standard.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 7th ed. Statistical Analysis-2 Limits of detection and determination, Statistical Analysis-4 Youden unit block collaborative testing procedure. The Society, St. Paul, MN, 1976.
2. Foster, A. Alternative method for analysis of total protein using the Nitrogen Determinator. *J. Am. Soc. Brew. Chem.* 47:42, 1989.
3. Guidelines for collaborative study procedures. *J. Assoc. Off. Anal. Chem.* 71:161, 1988.
4. Sweeny, R. Generic combustion method for determination of crude protein in feed: Collaborative study. *J. Assoc. Off. Anal. Chem.* 72:770, 1989.

APPENDIX

TOTAL NITROGEN IN BREWING GRAINS BY COMBUSTION METHOD

The combustion method for the analysis of total nitrogen measures nitrogen formed during the combustion of the sample in pure oxygen. The nitrogen gas is measured with a thermal conductivity detector. Total nitrogen is then calculated based on the response from a known organic nitrogen standard (2,3).

Reagents

(a) *Nitrogen standard*, ethylenediaminetetraacetic acid (EDTA) at 9.59% N.
(b) *Oxygen*, ultrapure grade.
(c) *Helium*, nitrogen-free.

Apparatus

(a) *Combustion nitrogen analyzer with thermal conductivity detector*, capable of reducing NO_x compounds to N_2.
(b) *Laboratory mill*, with 5-mm or smaller screen.
(c) *Analytical balance*, capable of 0.1-mg accuracy.

Method

Sample preparation. All samples must be milled before analysis (1-3).

Establish the instrument's baseline by running several reagent blanks.

Calibrate using EDTA (reagent a) by analyzing several repetitions and calculating the response factor.

Response factor. The response factor (RF) is calculated as

$$RF = [(9.59)\,(W_{std})]/[(A_s) - (A_b)]\,,$$

where
9.59 = % N in EDTA standard
W_{std} = weight of standard
A_s = area count of standard
A_b = area count of blank

Example

$W_{std} = 0.1501$
$A_s = 6{,}929$
$A_b = 35$

$$RF = [(9.59)\,(0.1501\text{ g})]/(6{,}929 - 35)$$
$$= 2.09 \times 10^{-4}$$

Analysis

Analysis of the samples is performed based on manufacturer's recommendations for sample size, furnace temperatures, burn times, flow rates, and other operational settings.

Calculation

Calculation of percent total nitrogen (%N) of the sample is usually performed by the integrator of the instrument, using the formula shown below. The formula assumes that baseline correction is available and used by the integrator. If baseline correction is not available, then the area of the blank will not be used in the calculation.

$$\%N = [(A_s - A_b) \times RF]/W_{sam}\,,$$

where
A_s = area counts of sample
A_b = area counts of blank
RF = response factor
W_{sam} = sample weight

Example

$W_{sam} = 0.1359$ g
$A_s = 3{,}709$
$A_b = 35$
$RF = 2.09 \times 10^{-4}$

$$\%N = [(3{,}709 - 35) \times (2.09 \times 10^{-4})]/0.1359$$
$$= 5.65$$

Precision

Based on a collaborative study of samples having total nitrogen contents of 1-5% (1), repeatability coefficients of variation of 1.3-5.5 and reproducibility coefficients of variation of 4.2-6.7 can be expected.

REFERENCES

1. American Society of Brewing Chemists. Report of Subcommittee on Total Nitrogen in Brewing Grains by Combustion Method. *Journal* 50:147, 1992.
2. Foster, A. Alternative method for analysis of total protein using the Nitrogen Determinator. *J. Am. Soc. Brew. Chem.* 47:42, 1989.
3. Sweeny, R. Generic combustion method for determination of crude protein in feed: Collaborative study. *J. Assoc. Off. Anal. Chem.* 72:770, 1989.