B.A. Johnson and C.-G. Johansson

Determination of the total soluble nitrogen content of malt and beer by the Dumas combustion method: collaborative trial

Submitted on behalf of the Analysis Committee of the Institute of Brewing and the Analysis Committee of the European Brewery Convention

The determination of the total soluble nitrogen content of malt and beer, by the Dumas procedure, has jointly been collaboratively tested by the Analysis Committees of the Institute of Brewing (IOB) and the European Brewery Convention (EBC). Five samples of beer (range 362 to 1159 mg/l) and five samples of malt (range EBC 0.598 to 0.798% m/m (dry basis) and IOB 0.534 to 0.706% m/m (dry basis)) were distributed to eighteen participating laboratories for analysis. Precision values were judged to be independent of the mean soluble nitrogen content for malt by both IOB and EBC methodologies. Values for r_{95} and R_{95} were 0.047 and 0.136%m/m for EBC laboratory wort and 0.039 and 0.144% m/m for IOB laboratory wort respectively. Precision values for beer were judged to be dependent upon the mean nitrogen content (m) in the case of r_{95} and independent of the mean nitrogen content in the case of R_{95}. Values for r_{95} and R_{95} were 0.074m and 120 mg/l respectively.

BC 34 Malt

(Descriptors: Soluble nitrogen, malt, beer, collaborative test.

Descriptoren: Löslicher Stickstoff, Malz, Bier, Ringuntersuchung).

1 Introduction

Developments in sophisticated instrumentation, which utilise the Dumas principle, together with continued concerns over the use of hazardous and toxic chemicals in Kjeldahl methodology has determined that the former has recently become a practical and popular alternative for the determination of nitrogen bound in organic matrices. The Dumas combustion method has been collaboratively tested and accepted by the feed industry in the United States of America (3), the American Society of Brewing Chemists (ASBC) for brewing grains (4), worts and beers (5), the IOB for barley and malt (6) and the EBC for barley and malt (7).

A collaborative trial was arranged under the auspices of the IOB and EBC Analysis Committees in order to establish precision values for the determination of the total soluble nitrogen content of beers and IOB and EBC laboratory worts.

2 Experimental

The organisation of the trial and statistical treatment of the data were carried out according to the procedures given in the International Standard ISO 5725 (8).

A uniform experimental design was employed and five samples of beer and five samples of malt were distributed to eighteen laboratories representing the brewing industry, the malting industry, research establishments and scientific instrument suppliers.

Participating laboratories were from the following companies: Pauls Malt Ltd, AB Pripps Bryggerier, Whitbread plc, Guinness Brewing GB, Malteries Franco-Belges, Brasseries Kronenbourg, VTT (Biotechnology and Food Research), Cargill Malt, Scottish Courage Brewing Ltd, Elementar GmbH, J P Simpson & Co. (Alnwick) Ltd., Muntons plc, R Kilgour & Co. Ltd., Carlsberg A/S and Crisp Malting Ltd.

Participants were requested to carry out analyses in duplicate and under repeatability conditions using the methods provided. The level of participation for each laboratory was dependent on their experience with the IOB and EBC methods for laboratory mashing. Instructions were provided for beer sample degassing.

Beer (9,10)

Determination of total soluble nitrogen	IOB Method 9.34.1 EBC Method 9.9.2

Malt EBC (9)

Malt mashing + sample preparation	EBC Method 4.5.1
Determination of total soluble nitrogen EBC	EBC Method 4.9.3
Determination of moisture content	EBC Method 4.2
Determination of extract	EBC Method 4.5.1

Malt IOB (10)

Malt mashing + sample preparation	IOB Method 2.4 (EBC Method 4.6)
Determination of total soluble nitrogen IOB	IOB Method 2.11.1
Determination of moisture content	IOB Method 2.3
Determination of extract	IOB Method 2.4 (EBC Method 4.6)

Table 1 Results of beer analysis (mg/l)

Lab No.	Beer 1		Beer 2		Beer 3		Beer 4		Beer 5	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Lab 1	1.102	1.129	635	658	461	471	557	553	363	386
Lab 2	1.184	1.187	686	673	498	495	610	599	381	375
Lab 3	1.230	1.260	720	700	530	530	630	620	350	370
Lab 4	††1.372	††1.331	717	717	474	464	**684	**573	340	300
Lab 5	1.189	1.099	644	654	473	507	536	565	363	373
Lab 6	1.149	1.168	664	654	488	484	587	585	364	350
Lab 7	†1.050	†980	570	620	460	480	**440	**510	390	370
Lab 8	1.150	1.220	730	690	520	490	590	600	380	380
Lab 9	1.180	1.180	680	680	480	470	580	580	350	340
Lab 10	1.160	1.200	710	780	530	530	620	620	400	390
Lab 11	1.178	1.171	655	609	473	466	573	543	337	328
Lab 12	1.140	1.130	658	660	565	560	430	427	‡	‡
Lab 13	1.186	1.204	696	678	503	504	614	592	359	370

** Rejected as Cochran's test statistical outlier on differences ($p \leq 0.01$)

†† Rejected as Dixon's test statistical outlier on means ($p \leq 0.01$)

† Identified as Dixon's test straggler on means ($p \leq 0.05$)

‡ No results returned

Table 2 Summary of precision data for beer

	Beer 1	Beer 2	Beer 3	Beer 4	Beer 5
n	12	13	13	11	12
m	1.159	674	496	573	363
r_{95}	85.6	62.6	29.7	30.6	35.0
R_{95}	169.9	122.5	86.1	155.1	66.0

Table 4 Summary of precision data for EBC malt

	Malt 1	Malt 2	Malt 3	Malt 4	Malt 5
n	12	12	12	11	12
m	0.798	0.706	0.712	0.598	0.647
r_{95}	0.0419	0.0453	0.0806	0.0230	0.0421
R_{95}	0.1327	0.1288	0.1605	0.1191	0.1386

Table 3 Results of EBC malt analysis (% m/m dry basis)

Lab No.	Malt 1		Malt 2		Malt 3		Malt 4		Malt 5	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Lab 1	0.755	0.747	0.695	0.677	0.669	0.670	0.579	0.572	0.622	0.632
Lab 2	0.826	0.823	0.741	0.746	0.732	0.724	0.632	0.620	0.674	0.671
Lab 4	0.875	0.846	0.778	0.748	0.796	0.805	0.640	0.650	0.730	0.730
Lab 5	0.754	0.770	0.657	0.619	0.665	0.583	**0.482	**0.564	0.557	0.592
Lab 6	0.81	0.80	0.70	0.68	0.69	0.71	0.61	0.61	0.64	0.64
Lab 7	0.82	0.79	0.71	0.70	0.73	0.72	0.61	0.61	0.67	0.65
Lab 9	0.715	0.715	0.675	0.648	0.666	0.667	0.577	0.559	0.602	0.602
Lab 11	0.78	0.73	0.65	0.63	0.67	0.67	0.55	0.56	0.57	0.59
Lab 13	0.838	0.853	0.753	0.737	0.749	0.778	0.643	0.657	0.690	0.711
Lab 14	0.815	0.834	0.747	0.756	0.766	0.772	0.643	0.622	0.682	0.688
Lab 15	0.851	0.850	0.728	0.761	0.727	0.783	0.589	0.590	0.643	0.694
Lab 16	0.78	0.78	0.70	0.72	0.72	0.63	0.52	0.51	0.62	0.63

** Rejected as Cochran's test statistical outlier on differences ($p \leq 0.01$)

Table 5 Results of IOB malt analysis (% m/m dry basis)

Lab No.	Malt 1		Malt 2		Malt 3		Malt 4		Malt 5	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Lab 1	0.51	0.53	0.49	0.50	0.58	0.57	0.57	0.57	0.64	0.64
Lab 3	0.62	0.60	0.54	0.54	0.68	0.67	0.68	0.66	0.75	0.75
Lab 5	0.549	0.558	0.540	0.489	**0.603	**0.538	0.572	0.538	0.667	0.635
Lab 7	0.58	0.56	0.53	0.52	0.60	0.58	0.60	0.61	0.70	0.70
Lab 8	0.580	0.598	0.539	0.539	0.636	0.618	0.660	0.625	0.682	0.707
Lab 9	0.546	0.555	0.504	0.496	0.619	0.619	0.590	0.599	0.717	0.691
Lab 10	0.635	0.654	0.565	0.611	0.674	0.674	0.690	0.700	0.812	0.831
Lab 11	0.57	0.57	0.52	0.51	0.63	0.63	0.63	0.70	0.70	0.69
Lab 12	0.612	0.621	0.675	0.679	0.691	0.689	0.709	0.710	0.801	0.804
Lab 17	0.53	0.53	0.49	0.46	0.62	0.64	0.57	0.59	0.66	0.65
Lab 18	0.578	0.541	0.512	0.511	0.612	0.604	0.568	0.546	0.659	0.641

** Rejected as Cochran's test statistical outlier on differences ($p \leq 0.01$)

In addition, participants were asked to complete a short questionnaire and carry out a "performance check" as described in the draft method distributed. A glycine standard solution prepared at a nominal concentration of 0.1% m/V was provided as an additional calibration crosscheck.

3 Results and discussion

The raw data as received is presented in Tables 1, 3 and 5 for beer, EBC wort and IOB wort respectively.

Five sets of data were identified as outliers and were rejected prior to the calculation of the performance characteristics, summarised in Tables 2, 4 and 6 for beer, EBC wort and IOB wort respectively. One further set was identified as a straggler. Whilst the method called for the precision of each laboratory to be evaluated (coefficient of variation less than 2% for repeat analysis) it was decided not to omit participating laboratories failing to meet this criterion as it had been set on an ad hoc basis.

Precision values were judged to be independent of the mean soluble nitrogen content for malt, by both IOB and EBC methodologies, and dependent upon the mean nitrogen content (m) of beer in the case of r_{95} and independent of the mean nitrogen content of beer in the case of R_{95}.

The overall mean precision values are presented in Table 7. The results of analysis of the glycine standard solution are given in Table 8 together with information provided by the participants in response to the questionnaire.

Table 6 Summary of precision data for IOB malt

	Malt 1	Malt 2	Malt 3	Malt 4	Malt 5
n	11	11	10	11	11
m	0.574	0.534	0.632	0.622	0.706
r_{95}	0.0357	0.0467	0.0236	0.0568	0.0342
R_{95}	0.1102	0.1608	0.1079	0.1645	0.1761

Table 7 Overall summary of precision data

	Beer Soluble Nitrogen (mg/l)	EBC Malt TSN (% m/m) dry basis	IOB Malt TSN (% m/m) dry basis
No. of Laboratories	13	12	11
Range	362 to 1159	0.598 to 0.798	0.534 to 0.706
Repeatability (r_{95})	0.074m	0.047	0.039
Reproducibility (R_{95})	120	0.136	0.144

4 Conclusion

The Analysis Committees of the EBC and IOB judged the repeatability and reproducibility precision values for the determination of total soluble nitrogen in malt and beer, by the Dumas combustion method, to be acceptable. The relevant methods have been approved for inclusion in Analytica EBC and the IOB Recommended Methods of Analysis.

5 Zusammenfassung

Johnson, B.A., und Johansson C.-G.: Bestimmung des gesamtlöslichen Stickstoffgehalts in Malz und Bier nach der Dumas-Verbrennungsmethode: Ringanalyse — Monatsschrift für Brauwissenschaft 53, Nr. 3/4, 50 – 53, 2000

BC 34 Malz

Die Bestimmung des gesamtlöslichen Stickstoffgehalts in Malz und Bier nach dem Dumas-Prinzip wurde von den Analysenkomitees des Institute of Brewing (IOB) und der European Brewery Convention (EBC) in einer Ringanalyse untersucht. Fünf Bierproben (im Bereich 362 bis 1159 mg/l) und fünf Malzproben (im Bereich nach EBC von 0,598 bis 0,798% m/

Table 8 Summary of questionnaire returns and performance check analysis

Lab No.	Instrument	Standard Material	Sample Size	Performance Check Coeff Var (%)	Glycine test solution (%)	
Lab No.		Standard material	Sple size		Rep 1	Rep 2
Lab 1	FP-428	glycine	1.0 ml	1.31	0.1000	0.1030
Lab 2	FP-428	orchard leaves	4 ml	0.93		
Lab 3	FP-428	glycine	1.2 ml	1.03	0.0993	0.0997
Lab 4	FP-428	glycine	1.5 ml	1.99	0.0950	0.1040
Lab 5	FP-2000	EDTA	1.0 ml	4.44	0.0990	
Lab 6	Macro-N	TRIS	3 ml	1.30	0.0949	0.0941
Lab 7	FP-428	EDTA	0.36 g	1.30	0.0960	0.0970
Lab 8	FP-428	glycine	0.5 ml	0.61	0.1000	0.1000
Lab 9	FP-428	glycine	1.4 ml	0.79	0.0990	0.1000
Lab 10	FP-428	glycine	1.5 ml	6.80	0.0900	0.0880
Lab 11	FP-2000	glycine	1 g	2.41	0.1000	0.1000
Lab 12	FP-428	glycine	1.40 g	1.51		
Lab 13	Macro-N	TRIS	3 ml		0.0924	0.0976
Lab 14	FP-428	EDTA	1.5 ml	1.14	0.0870	0.0870
Lab 15	Macro-N	TRIS	5 ml	3.40	0.0992	0.0907
Lab 16	FP-428			1.10		
Lab 17	FP-2000	glycine	1.6 ml		0.0995	
Lab 18	Rapid-N		0.65 g	1.85	0.1080	0.1080

m (Trockensubstanz) und nach IOB von 0,706 % m/m (Trockensubstanz)) wurden zur Analyse in 18 Untersuchungen aufgeteilt. Die Genauigkeitswerte des löslichen Stickstoffs für Malz waren unabhängig vom durchschnittlichen Wert bei den IOB- und den EBC-Methoden. Die Werte für die Wiederholbarkeit (r_{95}) und die Reproduzierbarkeit (R_{95}) betrugen 0,047 und 0,136% m/m für die EBC-Würzeuntersuchung, bzw. 0,039 und 0,144% m/m für die IOB-Würzeuntersuchung. Die Genauigkeitswerte für Bier waren abhängig vom durchschnittlichen Stickstoffgehalt (m) bei r_{95} und unabhängig vom durchschnittlichen Stickstoffwert bei R_{95}. Die Werte für r_{95} und R_{95} waren 0,074m bzw. 120 mg/l.

Johnson, B.A., et Johansson C.-G.: Détermination de la teneur en azote total soluble total dans le malt et de la bière suivant la méthode de combustion de Dumas. Test inter-laboratoires — Monatsschrift für Brauwissenschaft 53, Nr. 3/4, 50 – 53, 2000

BC 34 Malt

La détermination de l'azote soluble total dans le malt et la bière suivant le principe de Dumas a été évalué par le Comité des Analyses de l'Institute of Brewing (IOB) et de l'European Brewery Convention (EBC) au cours d'un test inter-laboratoires. Cinq échantillons de bière (intervalle de 362 à 1159 mg/l) et cinq échantillons de malt (intervalle d'après EBC 0,598 à 0,798 % m/m sur matière sèche et suivant IOB de 0,706 % m/m sur matière sèche ont été répartis en 18 examens pour analyse. Les valeurs de précision de l'azote soluble du malt étaient indépendantes de la valeur moyenne des méthodes IOB et EBC. Les valeurs de la répétabilité (r_{95}) et de la reproductibilité (R_{95}) s'élevaient à 0,047 et 0,136 % m/m pour l'examen des moûts EBC réspectivement 0,039 et 0,144 % m/m pour l'examen des moûts IOB. Les valeurs de précision pour la bière étaient dépendantes de la valeur moyenne en azote (m) pour r_{95} et indépendantes de la valeur moyenne enazote pour R_{95}. Les valeur de r_{95} et R_{95} étaient réspectivement 0,074 mg/l et de 0,120 mg/l.

6 References

1. Dumas, A., Annales de Chimie **33**, 342, 1826.
2. Kjeldahl, J., Meddeleser Fra Carlsberg Laboratorium **2**, 1, 1883.
3. Sweeney, J., Journal of the Association of Official Analytical Chemists **72**, 5, 770, 1989.
4. American Society of Brewing Chemists. Report of Sub-Committee on Total Nitrogen in Brewing Grains by Combustion Method, Journal of the American Society of Brewing Chemists **50**, 4, 147, 1992.
5. American Society of Brewing Chemists. Report of Sub-Committee on Nitrogen in Wort and Beer by Combustion Method, Journal of the American Society of Brewing Chemists **51**, 4, 183, 1993.
6. Buckee, G.K., Journal of the Institute of Brewing **100**, 57, 1994.
7. Johansson, C-G., Journal of the Institute of Brewing **102**, 249, 1996.
8. Precision of test methods —Determination of repeatability and reproducibility for a standard test method by inter laboratory tests. ISO standard 5725-1986.
9. European Brewery Convention, Analytica-EBC, 1998.
10. Institute of Brewing, Methods of Analysis, 1997.