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SPECTROPHOTOMETRIC DETERMINATION OF MALT COLOUR

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The International Method for the determination of the colour of beer has been tested by members of the Analysis Committee of the European Brewery Convention on samples of wort produced from a laboratory extract of malt using methods EBC 4.4 and EBC 4.4.5. The method, which relies on the spectrophotometric determination of colour at 430 nm, on clarified worts, is recommended as the designated reference method in place of the current visual method using EBC colour discs. The change will take effect from 1st January 1996. It was judged that precision values were dependent on the intensity of the colour of the sample over the range 3.6 to 25.3 EBC units. Repeatability (r_{95}) and Reproducibility (R_{95}) values of $r_{95} = 0.18Mean - 0.28$ and $R_{95} = 0.13Mean + 0.46$ were obtained over this range.

Key words: *Collaborative trial, malt (analysis method for), colour (determination of)*

INTRODUCTION

The current EBC (European Brewery Convention) method[1] for the determination of the colour of malt is EBC 4.7—Colour (Visual Method) which relies on the visual determination of malt wort colour by means of a comparator apparatus with glass discs of the EBC colours. For the determination of the colour of industrial worts, beers and liquid adjuncts method[2] EBC 8.3 is used, in which the absorbance of the sample is measured at a wavelength of 430 nm and the colour obtained by multiplying by a factor of 25. In order to assess the suitability of the spectrophotometric method for wort colour the Analysis Committee of the EBC decided to collaboratively test the spectrophotometric method for the determination of the colour of malt worts.

EXPERIMENTAL

The organisation of the collaborative trial and the statistical treatment of the data were carried out according to the procedures given in the International Standard[3] ISO 5725. Three trials were undertaken. All the trials employed a uniform design experiment.

In the first trial malt samples, ranging in colour from 3.7 to 7.8 EBC units, were distributed. Participants were requested to prepare duplicate samples of laboratory wort from each sample of malt following EBC 4.4—Extract[4]. A Schleicher and Schuell No. 5971/2 filter paper was specified for filtration of the laboratory mash. Participants were requested to clarify hazy worts, which were defined as having an absorbance at 700 nm of greater than 0.039 × the absorbance at 430 nm, following the procedure detailed in the EBC Visual colour method[1] which is by the addition of Merck Kieselguhr and further filtration, followed by further filtration through a 0.2 µ membrane filter, if necessary. Participants were asked to determine the colour of the resultant wort by EBC 8.3 Colour (International method).

In a second trial a further six malt samples, ranging in colour from 3.5–22 EBC units, were distributed. Participating laboratories were again requested to prepare duplicate samples of wort from each sample of malt. Participating laboratories who were members of the Institute of Brewing (IoB) were requested to produce an additional series of laboratory worts from each malt sample following EBC 4.4.5[5]. A Schleicher and Schuell No. 5971/2 filter paper was specified for filtration of the laboratory mash. Participants were requested to clarify hazy samples by filtration through a membrane filter of 0.4–0.5 µ pore size, discarding the first 20 ml of filtrate through a new membrane. The type of filter was left to the choice of the participant, although participants were requested to report the pore size, composition and manufacturer and the absorbance of the sample at 700 nm after filtration.

In the third collaborative trial six malt samples and two beer samples were distributed. Participants were requested to prepare duplicate samples of wort from each sample of malt following EBC method No. 4.4 or 4.4.5—Extract. A Schleicher and Schuell No. 5971/2 filter paper was specified for the mash filtration step. A Millipore Millex HA 0.45 µ membrane filter was specified for the wort clarification step. Participants were requested to clarify hazy samples until the absorbance at 700 nm was less than 0.010A. Participants were requested to determine the colour of the twelve worts by the EBC method 4.7—Colour (Visual Method) and the twelve worts and the two beer samples by method 8.3—Colour (Spectrophotometric Method) in order that the precision of the two methods for malt wort colour could be compared. It was specified that the colour must be determined within 30 mins of the end of filtration. The analysis of the two beer samples was to enable the precision of the spectrophotometric determination to be calculated.

RESULTS AND DISCUSSION

In the first trial 9 laboratories returned results. A number of participants were unable to clarify the laboratory wort to meet the specified criteria of A700 nm < 0.039 × A430 nm. This adversely affected the precision obtained. It was reported that kieselguhr treatment prior to filtration through a 0.45 µ membrane filter, increased the haziness of the sample rather than reducing it. The results from the first trial are not reported due to the unreliability of the data.

Thirteen laboratories returned results for the second trial. Of these, 5 laboratories returned results for both mashing methods. Although there did not appear to be any difference in the mean colours obtained between the two mashing procedures, the difference in the number of result sets between the two procedures made calculation of statistical significance invalid. In the second trial it was observed that the criterion for acceptability (A700 nm must be less than or equal to 0.039 × A430 nm) was probably not the correct criterion to use since the value obtained at 700 nm increases with increasing colour. Therefore, for very pale worts the criterion could not be met. It was agreed by the EBC Analysis committee, after examination of the results of the second trial, that a set value of 0.010 A at 700 nm was more appropriate. It was also observed that the colour of the wort increased on standing after filtration and that the type of membrane used in the clarification stage seemed to affect the colour of the wort obtained. The precision values from the second trial were no better than those from the first trial and have not been reported. It was suggested from the results of the second trial that the presence of visible haze particles in the sample does not necessarily affect the precision of the method since excluding hazy samples from the statistics did not reduce the reproducibility value.

The raw data from the third trial is presented in Tables I, II and III. Ten laboratories returned results. A summary of the statistical treatment of the results is given in Tables IV, V and VI. One set of data was identified as an outlier and was rejected. The overall summary of precision values is given in Table VII.

TABLE I Raw Data as received for Trial 3, EBC 8.3—Colour (Spectrophotometric Method)—Malt Samples

Lab	Malt A		Malt B		Malt C		Malt D		Malt E		Malt F	
1	13.13	13.35	20.23	20.68	27.78	26.08	7.25	8.05	8.58	9.08	4.13	4.1
2	13.6	12.9	20.9	19	26	25.7	7.55	7.73	8.75	8.6	3.48	3.6
3	13.55	13.08	17.15*	15.95*	22.1	23.65	7.18	6.83	7.65	7.98	2.3	2.18
4	15	14	22	24	25	26	8	7.9	9.1	9	3.5	3.4
5	14	14	20	20	28	26	8.5	8.4	9.6	9.5	4.5	4.9
6	13.25	13.2	20.58	20.78	25.05	27.25	8.33	7.93	9.25	9.15	3.48	3.6
7	14.15	14.11	20.85	20.81	24.73	24.81	7.92	7.97	9.16	9.18	3.72	3.72
8	13.78	13.83	20.3	19.9	25.25	25.9	7.88	7.95	8.98	9.05	3.55	3.48
9	13.98	14.23	20.73	20.88	24.38	24.5	8.28	8	8.68	8.98	3.55	3.43
10	15.0	13.5	19.0	20.6	24.4	22.9	8	7.8	8.6	8.8	3.5	3.5

*Identifued as an outlier according to Dixon's test.

TABLE II Raw Data as received for Trial 3, EBC 8.3—Colour (Spectrophotometric Method)—Beer Samples

Lab	Beer G		Beer H	
1	15.2	15.25	9.55	9
2	15.45	-	9.05	—
3	14.53	14.38	8.48	8.25
4	—	—	9	9
5	16	16	9.3	9.4
6	14.93	—	8.82	—
7	15.12	15.12	8.87	8.88
8	15.18	15.17	9.05	9.05
9	15.03	15.05	8.73	8.8
10	15.3	15.2	9.0	8.9

TABLE III Raw Data as received for EBC 4.7—Colour (Visual Method)—Malt Samples

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F	
1	—	—	—	—	—	—	—	—	—	—	—	—
2	12.81	12.81	16	16.5	23	24	8.25	8.13	8.25	8.5	2.87	2.81
3	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—
5	14	13	22	24	25	25	8.4	8.4	9.5	9.5	3.8	3.8
6	12	12	18	18	24	24	7.6	7.4	8.3	8.2	3.8	3.8
7	11.3	11.3	17.5	17.5	23.8	23.8	6.9	6.9	8.1	8.1	3	3.1
8	13.5	13.5	20.8	20.4	23.8	23.3	7.3	7.4	8.3	8.1	3.2	3.2
9	11.6	11.6	17	17	24	24	7.2	7.2	8.6	8.7	3.4	3.4

TABLE IV. Summary of Precision Data for EBC 8.3—Colour (Spectrophotometric Method)—Malt Samples

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
n	10	9	10	10	10	10
Mean	13.8	20.6	25.3	7.9	8.9	3.6
r_{95}	1.3	2.2	2.7	0.7	0.5	0.3
R_{95}	1.6	3.1	4.3	1.2	1.3	1.8

TABLE V. Summary of Precision Data for EBC 8.3—Colour (Spectrophotometric Method)—Beer Samples

	Beer G	Beer H
n	7.0	8.0
Mean	15.2	9.0
r_{95}	0.1	0.4
R_{95}	1.3	0.9

A number of participants reported that they were unable to clarify the worts to meet the clarity criterion specified (A700 nm must be less than or equal to 0.010). All samples met the criterion A700 nm less than 0.020A.

For the Spectrophotometric Method, with the exception of sample F, the precision is strongly dependent on the colour of the sample. For method 4.7, although repeatability (r_{95}) was found to be dependent on the mean value, no such relationship could be established for reproducibility (R_{95}). Reproducibility has therefore been calculated as the mean of the reproducibility obtained of all samples.

Overall the repeatability of the Visual Method—method 4.7 was better than that of the Spectrophotometric Method, but the reproducibility of the Spectrophotometric Method was better than that of the Visual Method. The Spectrophotometric Method gave higher mean colour values than the Visual Method. The reason for the improved repeatability of the Visual Method is probably a function of the poor sensitivity of the method.

Conclusion

The Analysis Committee of the EBC judged as acceptable both the repeatability and reproducibility values for the

TABLE VI. Summary of Precision Data for EBC 4.7—Colour (Visual Method)—Malt Samples

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
n	6	6	6	6	6	6
Mean	12.5	18.7	24.0	7.6	8.5	3.3
r_{95}	0.8	1.7	0.9	0.2	0.2	0.1
R_{95}	2.8	7.4	1.7	1.7	1.5	1.1

TABLE VII. Overall Summary

	EBC 8.3 Spectrophotometric Method Malt	EBC 8.3 Spectrophotometric Method Beer	EBC 4.7 Visual Method Malt
Range	3.6–25.3	9.0–15.2	3.3–24.0
r_{95}	0.18Mean–0.28	0.25°EBC	0.04Mean–0.04†
R_{95}	0.46 + 0.13Mean	0.32 + 0.065Mean	1.8°EBC†

†Sample B excluded.

determination of malt colour by EBC 8.3—Colour (Spectrophotometric Method). The committee has approved the method for inclusion in the malt section of Analytica-EBC and recommends that it is designated the reference method for the determination of malt wort colour because of its improved reproducibility over the visual method.

References

1. European Brewery Convention, ANALYTICA-EBC, 1987, Method 4.7.
2. European Brewery Convention, ANALYTICA-EBC, 1987, Method 8.3.
3. International Standard, ISO 5725, PRECISION OF TEST METHODS, second edition, 1986.
4. European Brewery Convention, ANALYTICA-EBC, 1987, Method 4.4.
5. European Brewery Convention, ANALYTICA-EBC, 1987, Method 4.4.5.