

Polyphenols in Beer

Subcommittee Members: H. S. Gress, *Chairman*; V. M. Bendelow, M. Dadic, R. L. McAdam, M. A. Mohs, G. Nickerson, W. G. Schulze, C. M. Tebeau, J. Whitt, and C. Hahn (*ex officio*).

CONCLUSIONS

Since a low total laboratory error (S_r) was observed in this collaborative study, the EBC Method of Analysis for total polyphenols appears to be a valid method for comparative studies of polyphenol levels.

RECOMMENDATIONS

It is recommended the method be investigated for at least one more year using a larger variety of lagers and ales.

Polyphenols in beer have been determined by a variety of methods, such as UV detection, ferric iron complexing, and binary analysis. Each analysis is specific for a certain polyphenol, or a combination thereof.

Three methods were considered for Subcommittee analysis. Harris and Ricketts (4) call for the adsorption of the anthocyanogen group on Nylon-66 resin. The method was time-consuming, but apparently gave consistent results ($\pm 5\%$). Another method, by M. Dadic (2), lends itself basically to the research field. This method is the binary analysis for determination of the two major groups of polyphenols: anthocyanogens and catechins. The procedure is lengthy, and does not lend itself to routine analysis.

The third method considered was the EBC colorimetric method developed by DeClerk and Jerumanis, reported by Bishop (1).

The present Subcommittee was assigned the evaluation of the EBC Method of Analysis for polyphenols. Nine collaborators analyzed one pair of samples consisting of two cans of beer from two consecutive lots of beer. The data were then accumulated and analyzed according to the Youden Unit-Block Design (5).

TABLE I
Collaborative Polyphenol Data

Collaborator	Total Polyphenols	
	Sample A mg/l.	Sample B mg/l.
1	118.1	118.1
2	122.2	108.8
3	111.9	111.9
4	118.1	105.0
5	113.6	113.2
6	111.0	116.6
7	119.0	115.0
8 ^a	99.0	97.0
9	118.0	117.0
Mean	116.5	113.2
Std. dev.	3.89	4.50

^aIdentified as an outlier (1), data not used in calculations.

TABLE II
Statistical Summary of Polyphenol Data

Sample	Method	No. Labs	Grand Mean ($\bar{X}+F$)/ Z^2	Within-Lab Error S_1	Between-Lab Error S_0	Total Error S_1^2/S_0^2	Calc. F Ratio 95%	Critical F S_0	Coefficient of Variation
Pair I	EBC	8	114.9	4.73	0.00	3.60	0.58	3.787	3.13

^amg/l.

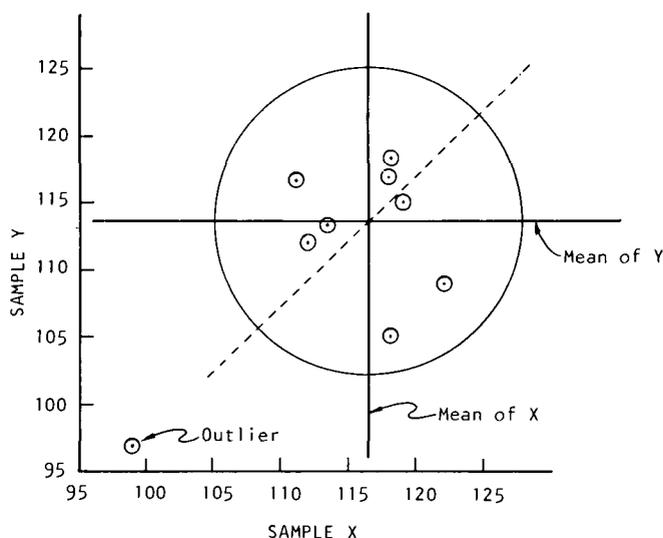


Fig. 1. Two-sample chart for polyphenols.

PROCEDURE

The collaborators were asked to run the analyses on each pair of samples using the EBC Method of Analysis.

The method was originally reported by DeClerk and Jerumanis [cf. Wallerstein Lab. Commun. 31, No. 106, p. 225 (1968)] and was adopted by the European Brewing Congress after collaborative study. The method is a colorimetric analysis using a ferric reagent which is nonspecific for phenolics. The sample to be analyzed must be clear. Ten milliliters of the sample, 8 ml of CMC/EDTA reagent (1% solution of low-viscosity carboxymethylcellulose, 0.2% disodium ethylenediamine tetraacetate), 0.5 ml of 3.5% ferric ammonium citrate, and 0.5 ml ammonia reagent (concentrated ammonia diluted with two volumes of distilled water) are mixed in

order, shaking between each addition. The solution is then made up to 25 ml with distilled water, shaken, and allowed to stand for 10 min. The absorbance is measured at 600 nm in a 1-cm cell against a blank treated similarly except for the omission of the ferric reagent. The polyphenol concentration in mg/l. is then given by the $A_{600} \times 820$.

RESULTS AND DISCUSSION

Tables I and II show the statistical breakdown of the data obtained for the pair of samples observed. One set of polyphenol data was rejected since the values obtained were outliers according to Dixon's test (3). The data were subjected to an analysis of variance according to Youden; the results are recorded in Table II. Figure 1 is a two-sample chart representing the data obtained from the collaborators.

The calculated F ratio was much smaller than the critical F value indicating a lack of systematic error. The within-laboratory error (S_1), from Table II, shows a 4.73 variability. After removing the S_1 value, a 0.0 value was attained for the between-laboratory error (S_0). A high between-laboratory value would indicate a shortcoming in the method. The coefficient of variation was 3.13 indicating, again, a satisfactory analysis for comparative testing.

The method is not one intended to give an absolute value for polyphenol content. It is, however, one that is relatively quick, simple, and, in view of the present study, precise. This makes it an advantageous method for routine analysis. It appears to be a good method for a reference point: a relative value for comparative or control purposes.

It is recommended that the method be used on a larger variety of lagers and ales, thus investigating its validity over a range of polyphenol content.

Literature Cited

- BISHOP, L. R. *J. Inst. Brew.* 78: 37 (1972).
- DADIC, M. *Amer. Soc. Brew. Chem., Proc.* 1971, p. 149, 159.
- DIXON, W. J. *Biometrics* 9: 74 (1953).
- HARRIS, G., and RICKETTS, R. W. *J. Inst. Brew.* 65: 331 (1959).
- YOU DEN, W. J. *Statistical techniques for collaborative tests*, p. 27. *Ass. Offic. Anal. Chem.*: Washington, D.C. (1969).