Automated Procedure for the Estimation of Total Polyphenol Content in Beer, Wort, Malt, and Barley[1]

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ABSTRACT

An automated procedure using 4-aminoantipyrine as a chromogen was used to estimate total polyphenol content in beer, wort, malt, and barley. For 23 beers with a wide range in test results, the correlation between the manual European Brewery Convention method and the automated method was 0.88. Barley and malt determinations were made on 30% aqueous dimethylformamide extracts of grain. Cultivar differences were found, suggesting the use of the procedure to select for polyphenol content in lines from barley breeding programs.

Key words: *Automated analysis, Barley, Beer, Polyphenols.*

Suggested methods for the estimation of total polyphenol content in wort and beer are based on the ability of phenolic compounds to form colored products on reaction with acids, oxidizing agents, certain metals, and diazotized amines. The recommended method of the European Brewery Convention (2) uses iron citrate as a chromogen. Dadic's procedure (3) is based on the red color produced by reaction with butanol-hydrochloric acid. Singleton (6) suggested the use of a Folin-Ciocalteu reagent in beer analysis and Woof and Pierce (7) described an automated procedure based on diazotized *p*-amino-benzoic acid. Of many available reagents, Macfarlane (4) selected 4-aminoantipyrine because of its high sensitivity and reasonable specificity for phenols. This reagent was also the choice of Ng and Mocek (5), who reported minimal interference by proteins and carbohydrates.

This paper describes a simple, reliable, automated method using 4-aminoantipyrine for estimating total polyphenol content in beer, wort, malt, and barley.

MATERIALS AND METHODS

Samples

Samples of Canadian beers were supplied by Winnipeg breweries and single bottles of imported beers were purchased from the

[1]Contribution No. 797 from Agriculture Canada, Research Station, 195 Dafoe Road, Winnipeg, Manitoba, R3T 2M9.

Manitoba Liquor Control Commission. Beers were decarbonated by ASBC method **BEER–1** (1) before analysis. Worts were prepared by ASBC method **MALT–4** (1) except that additional malt:water ratios were used to provide a range in extract concentration. Barley samples were grown in field trials in western Canada in 1976 and malts were made in laboratory equipment. Ground barley and malt samples (5 g, dry basis) were extracted with 50 ml of 30% (v/v) aqueous dimethylformamide (DMF) at 22°C for 45 min on a slow-speed Eberbach shaker. The mixture was filtered through Whatman No. 1 paper. It was occasionally necessary to clarify barley extracts by centrifugation.

Analysis

Total polyphenol contents of beer, wort, and DMF extracts of barley and malt were estimated using the autoanalyzer arrangement shown in Fig. 1. The reagents used were:

1. 20 ml of ammonia solution (sp gr 0.90) diluted to 100 ml with water. For beer and wort tests, this solution contained 0.1% disodium ethylenediamine tetraacetate to prevent formation of a precipitate.
2. 0.3% (w/v) 4-aminoantipyrine in water. This solution was prepared at least 1 hr before use and replaced every 2 days.
3. 0.6% (w/v) potassium ferricyanide in water, replaced every 2 days.

Sampling rate was at 60/hr and transmittance was read at 505 nm. The colorimeter std cal control was used to sensitize the analyses; in this work, the std cal setting was 580 for beer and wort and 350 for barley and malt. A blank reading was determined for wort and beer by processing samples with ammonia but without the other two reagents. Standards were not included in the blank run. The difference in transmittance between reagent and blank was used in calculating results.

The procedure was standardized with solutions of gallic acid. A stock solution of 0.276 g of gallic acid monohydrate in 250 ml of water was prepared and aliquots diluted to 100 ml. Beer and wort standards contained 10 and 20 ml of stock in 100 ml of water (equivalent to 100 and 200 mg/l). Barley and malt standards were 25 and 35 ml of stock diluted to 100 ml after adding 30 ml of DMF (equivalent to 0.25 and 0.35%). New standards were made when the stock solution showed slight discoloration. The use of gallic acid, which reacts with ammonia alone to produce a colored product, was arbitrary. It has the advantages of high purity, ready solubility, and relatively good stability compared with other reagents, and its use in this procedure gave day to day comparable results.

Determinations of polyphenol content were made on beer and wort by the method of the European Brewery Convention (2). Results were expressed as mg/l., calculated by multiplying the

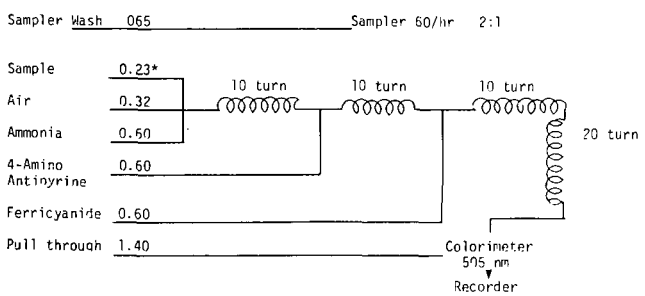

Fig. 1. Auto-analyzer diagram for total polyphenols

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TABLE I
Relation Between % Transmittance and Concentration

vol Diluted to 10 ml ml	100–% Transmittance		
	Beer 1	Beer 2 505 nm	Wort
2	16	9	11
3	24	14	
4	33	19	21
5	40.5	24.5	
6	49	30	32
7	58	35	
8	73	40	42
9	82	45	

TABLE II
Reproducibility

Aliquot	Polyphenol Content	
	Beer A	Beer B
	mg. gallic acid/l.	
1	131	210
2	137	204
3	136	202
4	132	208
Mean	134	206
Range	6	8
Std. dev.	2.9	3.6

TABLE III
Total Polyphenol Content of Beers by Automated and by EBC Methods

Sample Number	Country of Origin	Polyphenols	
		Automated	EBC
		mg/l.	
8	Denmark	90	73
1	USA	106	97
12	England	121	99
2	Japan	130	117
15	Canada	132	110
21	Canada	134	119
18	Canada	138	112
20	Canada	138	136
10[a]	Scotland	138	136
19[a]	Canada	145	151
13[a]	England	145	155
3	Holland	154	153
23	Canada	157	136
14	Canada	160	136
22	Canada	160	140
5	Philippines	167	153
9	Australia	170	147
4[a]	England	170	134
17	Canada	188	138
16[a]	Canada	192	144
7	Germany	198	173
11	Poland	206	173
6	Czechoslovakia	226	211

[a]Labeled as ale; other samples described as beer or lager.

TABLE IV
Total Polyphenol Content of Barley and Malt

Cultivar	% Polyphenol, as Gallic Acid	
	Barley	Malt
Betzes	0.245	0.250
Klages	0.265	0.275
TR201	0.310	0.320
TR203	0.290	0.295
TR428	0.280	0.290
TR320	0.235	0.245
TR907	0.285	0.305
TR910	0.275	0.290

TABLE V
Total Polyphenol Content of Worts

ml Water to 25 g Malt	Malt mg/ml	Polyphenol Content		EBC mg/l.
		Automated, as gallic acid		
		% malt	mg/l.	
420	60	0.112	67	47
330	76	0.114	87	56
280	89	0.111	99	67
210	119	0.111	132	88
170	147	0.113	166	109

difference in absorbance (1 cm cell) of test sample and blank by 820. In the automated test, results were expressed in terms of gallic acid:mg/l. for beer and wort and % of grain for barley and malt.

RESULTS AND DISCUSSION

The data in Table I are the transmittance at 505 nm (for convenience given as 100 minus % T) of aliquots of 2 to 9 ml of beer and ASBC wort diluted to 10 ml with water. The relation between concentration and transmittance is linear. Samples of two beers, stored cold, were decarbonated and analyzed on 4 consecutive days, using fresh reagents each time. The results (Table II) show that the automated method is satisfactorily reproducible.

Table III shows the total polyphenol content of 23 beer samples, determined by the automated and EBC methods. The correlation between the two values is 0.88 for all samples. Omitting samples 4, 10, and 13, which were dark ales with high blank readings, increased the coefficient to 0.92. After correction for the blank, the range in absorbance in the EBC test was 0.12. In the automated method, the range (calculated from % transmittance) was 0.48. These results suggest that the automated method is as effective as the EBC procedure in differentiating between beer samples and that it is more sensitive.

The range of polyphenol content of worts made from different malts at constant water:malt ratio was smaller than that of the beer samples. In ASBC worts prepared from malts made from the barley cultivars listed in Table IV, the polyphenol content range was 125 to 156 mg/l. A wider range was obtained by using other malt:water ratios and the results of automated and EBC tests on the worts are shown in Table V. The data show that the automated method differentiates between worts with respect to their polyphenol content.

The automated method was used to estimate the total polyphenol content of barley and malt. The data in Table IV are mean values for eight two-row barley cultivars grown at three locations. Cultivar differences are evident; Betzes and TR430 were low, and TR201 and TR907 were high in total polyphenols. These results suggest that the method is applicable in commercial analyses of barley and malt and may be particularly useful in the selection for optimal levels of polyphenol content in lines from malting barley breeding programs.

CONCLUSION

Many of the reported methods for estimating polyphenol content are time-consuming, insensitive, or poorly reproducible. Attempts to automate the procedures were only partly successful due to the formation of precipitates, adsorption of reagents on analyzer components, etc. The method described was the only one of eight examined that, when automated, performed with complete satisfaction in every-day operation. All colorimetric polyphenol determinations are necessarily empirical, as the color yield may vary for different components of the polyphenol complex. Thus, the method appears to be as sound in principle as other procedures and has the advantages of rapidity, sensitivity, and reliability.

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