Automated Analysis of Total Polyphenols in Beer[1]

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ABSTRACT

Total polyphenols in several beer samples were determined using automated procedures, and the results obtained were compared with the ASBC method for total polyphenols. The automated method provided slightly lower polyphenol readings; however, the correlation coefficient between the two methods was 0.988. The automated method required minimal instrument set-up, standardization, and sample preparation, and used stable reagents. The automated procedure can analyze up to 40 samples per hour.

Brewing raw materials such as barley-malt husks and hops contribute phenolic and polyphenolic substances to the early stages of the brewing process. Further polymerization of these materials can occur during wort boiling and possibly during fermentation to give rise to the polyphenols found in beer. The presence of polyphenols in beer is important for several reasons; for instance, polyphenols furnish color, impart astringent taste, and serve as a reservoir for both oxygen reduction and substrate browning. In addition, these compounds assist in the agglutination of poorly coagulable proteins and protect against the formation of complexes between the bitter substances in hops. The role of complex phenolic compounds in brewing has been reviewed by Gardner and McGuiness (7), who considered the mechanisms of haze formation in detail and discussed the contribution of phenols to other beer characteristics. As polyphenols play an important role in beer stability, there exists a need within the brewing industry for a rapid, accurate, and sensitive method for measuring total polyphenols in beer.

Literature references (3,7) show several techniques for estimating the total polyphenols in beer. Most the the manual methods reported in the literature are time-consuming, insensitive, and exhibit poor reproducibility. These procedures usually require separation of polyphenols from sample matrixes either by precipitation with heavy metals or extraction with organic solvents. Separated polyphenols are then analyzed either by a physical or by a wet chemical analytical procedure. In the wet chemical analysis, polyphenols are allowed to react with acids, oxidizing agents, metal salts, or diazotized amines to form colored products (3), the intensity of which is then measured colorimetrically. Polyphenols from beer have also been separated by employing Sephadex or polyacrylamide (8,10) and analyzed by using high-performance liquid chromatographic (5) procedures.

Dadic's method (6) treats polyphenols with butanol and hydrochloric acid to generate color. Woof and Pierce (11) developed a semiautomated procedure based on the reaction of polyphenols with diazotized *p*-aminobenzoic acid. McFarlane (9) used 4-aminoantipyrene as color-forming reagent for polyphenol. The ASBC (1) adopted a method that utilizes iron citrate as a chromogen for analyzing a wide range of polyphenols in beer. This paper describes a semiautomated procedure for measuring total polyphenols using chemical reagents as described by the ASBC total polyphenol method. The method is simple, reliable, and capable of analyzing 40 samples an hour.

EXPERIMENTAL

Apparatus

The analyzer was a Technicon AutoAnalyzer II (AAII) with a continuous flow analytical system. A sample IV, proportioning pump III, and a cam set at 40 samples/hr with a 5:1 sample-to-wash ratio were used. The colorimeter was equipped with a 15-mm flow cell and 600-nm filters. A voltage stabilizer, recorder, and AAII glassware and fittings were used.

Reagents

Distilled water was used exclusively for reagent preparation. Chemicals used were ACS reagent grade.

Complexing solution. The solution was made by adding 2.0 g of carboxymethyl cellulose (CMC) to a 1-L volumetric flask containing 10 ml of ethyl alcohol and a magnetic bar. The flask was shaken to partially dissolve the CMC, and 15.0 g of disodium ethylenediaminetetraacetate (EDTA) and 800 ml of water were added. The mixture was stirred until the contents dissolved, and 4 ml of of Aerosol-22 (Technicon No. T21-0300, Tarrytown, NY) was added. The contents were diluted to volume with water. The reagent was stable for one month.

Ferric ammonium citrate. A 2%, w/v, aqueous solution was made by dissolving 20.0 g of ferric ammonium citrate in about 800 ml of water. One milliliter of Aerosol-22 was added and the contents diluted to volume (1 L) with water. The reagent was kept in a brown bottle and made fresh each week.

Ammonium hydroxide. An aqueous solution (2%, w/v) was made by dissolving 20 ml of concentrated ammonium hydroxide solution in about 800 ml of water. The contents were diluted to 1 L with water.

Standard Solutions

Stock standard. The stock standard was a 0.4%, w/v, aqueous gallic acid solution made by dissolving 0.4 g of gallic acid in about 80 ml of water. The contents were diluted with water to 100 ml. The reagent was stored in a brown bottle in the refrigerator.

Working standard. Working standard solutions were prepared at concentrations of 40, 80, 120, 160, and 200 mg/L of gallic acid by pipetting 1, 2, 3, or 5 ml, respectively, of stock standard into 100-ml volumetric flasks and diluting the contents to volume with water. The working solutions were stored in brown bottles in the refrigerator.

Preparation of sample assay. About 50 ml of beer sample was transferred into a beaker containing a magnetic stirring bar. The sample was stirred for about 5 min to remove dissolved gasses. The degassed beer sample was poured into plastic sample cups for analysis.

Analysis of sample. The manifold (Fig. 1) was constructed from the AAII modules. All reagent lines were placed in their respective containers. The proportioning pump was started, and the system was left running for about 10 min to attain equilibrium. Working standards and samples were transferred to plastic sample cups and were placed in the Sampler IV. The reagent baseline absorbance with respect to water was in the range of ±0.04–0.06%.

Methodology Notes

Multiple working standards were used to establish linearity for routine operation; a midscale standard (120 mg/L) was used for instrument calibration. Water containing Aerosol-22 was used for wash in the system. To obtain sample blank readings, the ferric ammonium citrate reagent line was transferred to water and the samples were rerun. The system was washed by placing all reagent lines in water containing Aerosol-22 reagent. To remove coating on the mixing coils, 2*N* HCl was pumped through all the reagent lines for about 10 min. HCl was removed from the system by pumping water through the reagent lines before analyzing samples.

[1]Presented at the 48th Annual Meeting, Kansas City, MO, May 1982.

Calculation

The intensity of the color, which is proportional to the concentration of gallic acid in solution, was recorded on the chart paper. The amount of polyphenols present in the sample was calculated by following the procedure described by Bishop (4).

RESULTS AND DISCUSSION

The manifold in Figure 1 shows the chemical reaction sequences involved in the color development in the present procedure. The effects of several variables were examined for their influence on the color formation and quantitative applications of the reaction. The factors included the stability and intensity of color, reagent concentrations, final pH, time for color development, filter wavelength, conformity to Beer's law, and interference from other compounds.

Degassed samples were added to an air-segmented reagent line containing CMC and EDTA. Ferric ammonium citrate and ammonium hydroxide were added to the segmented stream. The resulting red color was measured colorimetrically at 600 nm. Sample blank readings were obtained by replacing the ferric ammonium citrate reagent line with water and rerunning the samples. The rate of analysis was 40 samples/hr. The time required from introduction of sample into the system to read out on the chart paper was about 8 min. The callibration curve was determined for gallic acid by plotting absorbance against concentration. Figure 2 shows the straight-line calibration curve obtained from gallic acid in the range of concentrations studied during this investigation.

The effects of varying amounts of ammonium hydroxide and ferric ammonium citrate on the intensity of color formation were examined by analyzing beer and beer spiked with 50 ml/L of gallic acid. Figure 3 shows the influence of ammonium hydroxide on color development; increasing the strength of ammonium hydroxide above a 2% solution causes a gradual decrease in sensitivity. Variations in the amount of ferric ammonium citrate (Fig. 4) from 1 to 4% did not produce any significant change in color absorbance. However, when ferric ammonium citrate in the range below a 1% solution was used, a gradual decrease in sensitivity was observed. A solution of 2% EDTA, 1% ferric ammonium citrate, and 2% hydroxide was chosen as the optimum reagent system (the final pH of the reaction mixture was 12) for analyzing beer by the automated procedure. These reagents, when added to a sample in the order shown in the manifold (Fig. 1), gave the optimum color intensity in the system.

In addition to gallic acid, several other phenolic compounds were studied for color development. Results are given in Table I. Among the compounds examined, pyrogallol gave an absorbance equal to that obtained from gallic acid. Phloroglucinol reacted to give insignificant color. Results in Table I show that the red color is obtained only with phenolic compounds having three vicinal -OH groups.

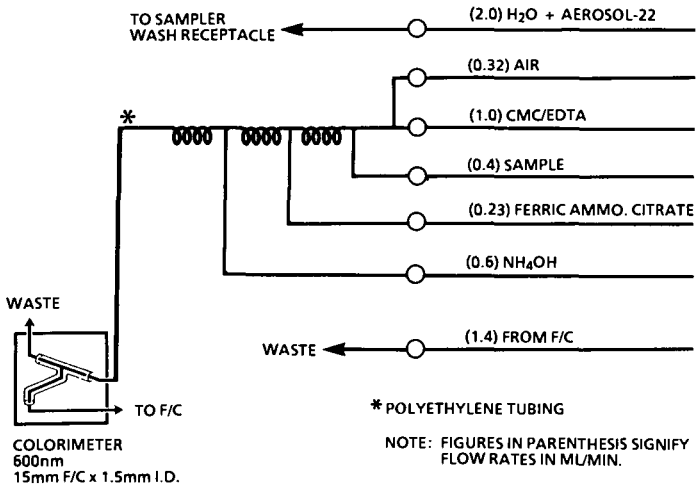

Fig. 1. Flow diagram for semiautomated analysis of total polyphenols in beer.

TABLE I
Color Development With Phenolic Compounds[a]

Compound	Amount Used (mg/L)	Absorbance
Gallic acid	0.117	0.39
Pyrogallol	0.126	0.39
Phloroglucinol	0.162	0.05
Resorcinol	0.110	0.00
Vanillin	0.152	0.00
Phenol	0.103	0.00
O, O'-Biphenol	0.186	0.00
Orcinol	0.124	0.00
Salicylaldehyde	0.122	0.00

[a]The amounts used represent equimoles of phenolic substances examined.

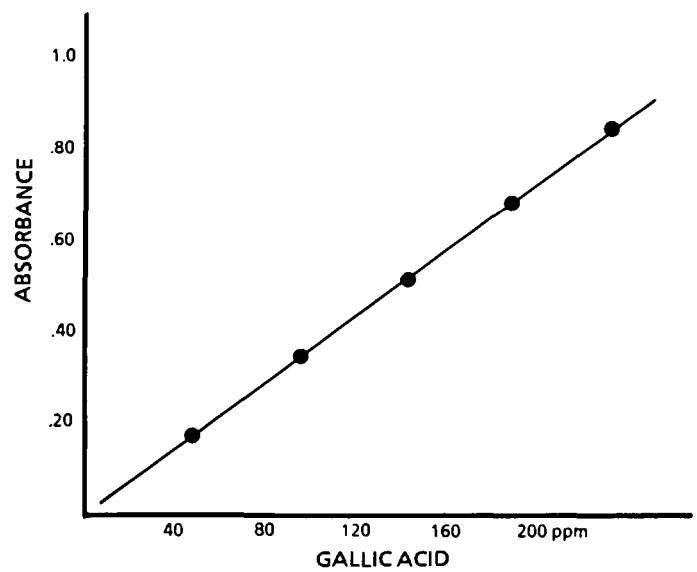

Fig. 2. Linearity of the method.

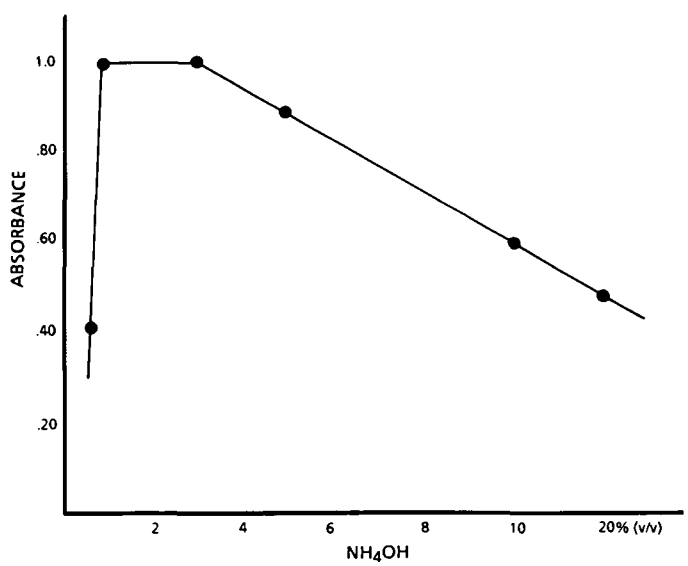

Fig. 3. Effect of change in NH_4OH concentration on color formation

Several other organic and inorganic bases besides ammonium hydroxide were examined for their effects on color development. Results obtained are recorded in Table II. Among the organic bases tested, triethylamine gave the highest sensitivity. Sodium carbonate also gave higher sensitivity in comparison to ammonium hydroxide. However, the use of selected inorganic bases in place of ammonium hydroxide caused precipitation of the reagents in the flow cell. The use of organic bases produced noisy peaks and drift in baseline.

The within-run precision of the automated method was determined by analyzing 40 replicates of 120 mg/L standard gallic acid solution. The coefficient of variation was ± 1.5%.

The accuracy of the present procedure was determined by comparing the values of polyphenols found by the automated method with those found by the ASBC total polyphenols method. The results are tabulated in Table III. Several categories of beer were obtained from retail outlets for a total of 53 samples analyzed for total polyphenols by the automated and ASBC methods. In general, the automated procedure gave slightly lower results in comparison to the ASBC method.

The linear regression line (Y = 0.9925, X = 8.8137) computed from experimental data, obtained by the automated (X) versus those obtained on identical samples by the ASBC method (Y), had a Y-intercept of +8.8137 and a slope of 0.9925. The X coefficient should be one for equivalent response curve, and Y-intercept should be zero for the equivalent response to polyphenols endogenous to beer. The correlation coefficient for the two methods was 0.9879. The standard error of estimate was 3.5420. The correlation between the automated and the ASBC methods is shown graphically in Figure 5.

Recovery studies were performed on two groups of beer samples. An aliquot of beer sample was spiked with a known

TABLE II
Color Reaction With Organic and Inorganic Bases

Base	Amount Used[a]	Absorbance
Ammonium hydroxide	2	0.45
Diethylamine	2	0.45
Triethylamine	2	0.62[b]
Triethanolamine	2	0.09
Pyridine	2	0.02
Sodium carbonate	1	0.04
Sodium bicarbonate	1	0.65[c]
Sodium hydroxide	1	0.35[c]

[a]Organic bases used were 2%, v/v, aqueous solution. Inorganic bases used were 1%, w/v, aqueous solution, except ammonium hydroxide which was 2%, v/v, aqueous solution.
[b]Noisy peaks and drift in baselines were observed.
[c]Coating in transmission tubing and flow cell occurred. Results were not reproducible.

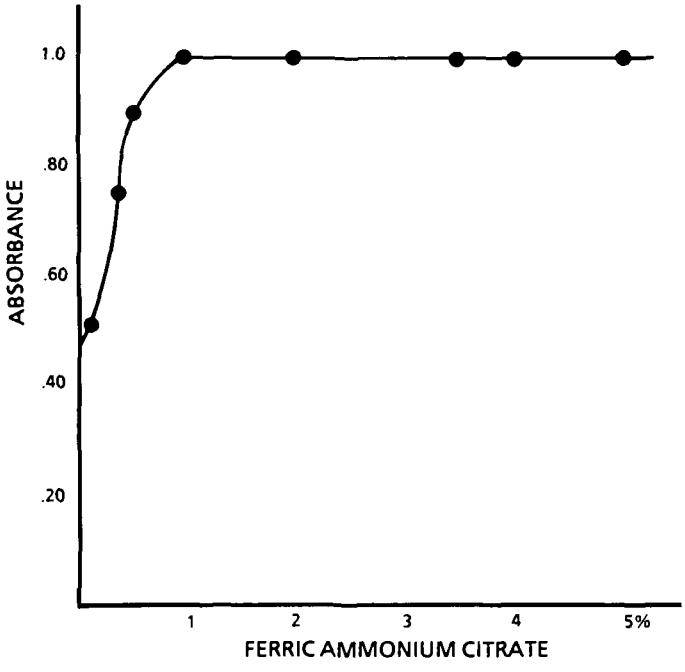

Fig. 4. Effect of change in concentration of ferric ammonium citrate on color formation.

TABLE III
Comparison of Automated Assay With Manual ASBC Values of Total Polyphenols[a]

Sample No.	Automated Assay (mg/L)	ASBC Method (mg/L)
1	115.0	120.0
2	131.0	134.0
3	116.0	123.0
4	130.0	132.0
5	137.0	132.0
6	125.0	129.0
7	129.0	134.0
8	128.0	130.0
9	130.0	133.0
10	133.0	138.0
11	98.0	106.0
12	73.0	79.0
13	111.0	116.0
14	90.0	97.0
15	98.0	106.0
16	96.0	102.0
17	97.0	102.0
18	99.0	106.0
19	145.0	154.0
20	168.0	175.0
21	153.0	163.0
22	173.0	185.0
23	169.0	181.0
24	162.0	170.0
25	162.0	169.0
26	152.0	158.0
27	162.0	172.0
28	123.0	130.0
29	136.0	145.0
30	119.0	125.0
31	141.0	148.0
32	132.0	142.0
33	134.0	144.0
34	131.0	140.0
35	135.0	142.0
36	137.0	145.0
37	103.0	111.0
38	102.0	112.0
39	97.0	107.0
40	106.0	115.0
41	111.0	125.0
42	101.0	112.0
43	105.0	120.0
44	104.0	115.0
45	103.0	116.0
46	111.0	122.0
47	104.0	112.0
48	113.0	120.0
49	122.0	133.0
50	112.0	121.0
51	116.0	131.0
52	121.0	129.0
53	126.0	137.0
Average	123.0	131.0

[a]Results were calculated by following the procedure described by Bishop (4).

amount of gallic acid and analyzed. The recovery data are recorded in Table IV. An average of 99.4% of the added gallic acid was recovered.

Certain phenolic compounds, such as gallic acid and pyrogallol, have stronger affinity for metal ions, especially for Fe(III), and under special experimental conditions form stronger Fe(III)-phenol complexes than either Fe(III)-EDTA or Fe(III)-citrate complexes. In addition, in alkaline condition, the stability of Fe(III)-EDTA or Fe(III)-citrate is not high enough to keep it from decomposing and precipitating as $Fe(OH)_3$. Consequently, during the course of reactions in the ASBC total polyphenol method, Fe(III) ions are progressively complexed with phenolic substances and form colored Fe(III)-phenol complexes. Under certain experimental conditions, EDTA may also be incorporated to form a complex consisting of Fe(III), phenol, and EDTA in a 1:1 molar ratio. The presence of excess amounts of EDTA was desirable in the automated method for preventing interfering metal ions from precipitating out of alkaline solution as metal hydroxide. In addition, we found that the use of CMC in the present automated procedure was not critical to obtain reliable results and, if desired, might be omitted.

TABLE IV
Percent Recoveries of Gallic Acid Added to Beer[a]

Sample No.	Amount of Gallic Acid		% Recovery
	Added (mg/L)	Recovered (mg/L)	
1	10	9.86	98.6
2	20	18.26	91.0
3	30	27.5	92.0
4	40	39.1	97.8
5	50	50.2	100.4
6	60	61.0	101.0
7	70	71.7	102.0
8	80	83.0	103.0
9	100	105.0	105.0
10	10	9.88	98.8
11	20	19.76	98.8
12	30	28.9	96.3
13	40	38.5	96.2
14	50	50.7	101.0
15	60	60.3	100.5
16	70	69.9	99.8
17	80	80.3	100.3
18	90	93.0	103.3
19	100	103.0	103.3
Average			99.4

[a]Five milliliters of sample was spiked with increasing amounts of gallic acid and analyzed.

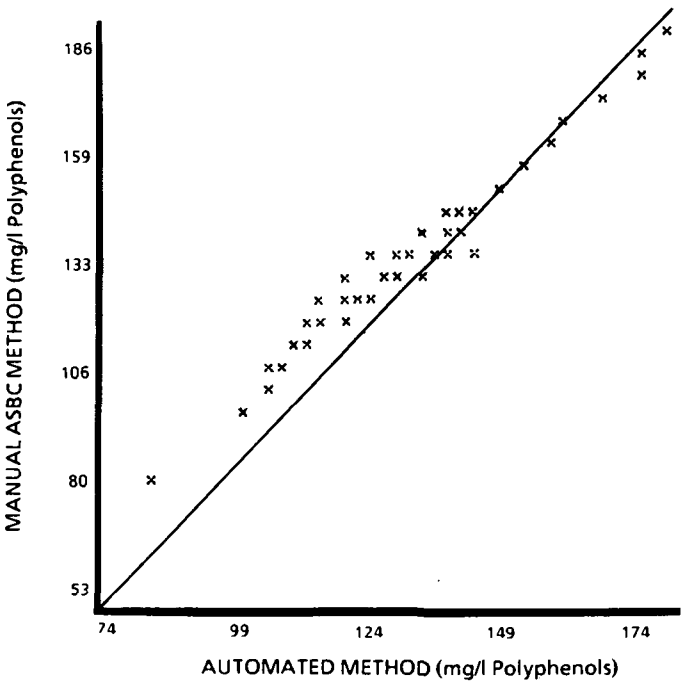

Fig. 5. Automated and manual ASBC assays compared for polyphenols in beer samples.

Usually phenolic compounds possessing at least two neighboring phenolic hydroxyl groups form a complex with metal ions in alkaline solutions. Where a compound contains these vicinal hydroxyl groups, e.g., pyrogallol and gallic acid, only two of the groups form complexes for steric reasons. A benzene ring also appears to be essential for formation of these complexes because glycerol, which has these hydroxyl groups attached to an aliphatic nucleus, does not give a similar reaction.

As colored complexes formed between polyphenols and Fe(III) ions are much more labile in ammoniacal solution than in NaOH or KOH solution, the use of ammonium hydroxide is essential in the present procedure.

Because polyphenols are unselective and react with most metal ions existing in a trivalent or higher state to form color, the present automated procedure may not be applicable as a reference method. However, its rapidity, sensitivity, and reliability, give the automated method an advantage for use in quality control and comparative laboratory analysis of polyphenols in beer.

CONCLUSION

The present automated procedure provides a rapid method for the determination of polyphenols in beer and exhibits good correlation with the presently accepted ASBC total polyphenol manual method. The method requires minimal set-up time, preparation, and operator skill. It employs stable and inexpensive reagents.

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