Evaluation of a Gallery Total Polyphenol Method Performance in Beer (and Wort) using the EBC / MEBAK protocol

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Abstract
The purpose of this study was to automate the EBC protocol to measure Total Polyphenols in beer and wort samples using automated Thermo Scientific Gallery analyzer. Results of the Gallery were compared to the results of commercial BABs reference samples. As a result of this study, for the 7 beer samples analyzed, the BABs assigned values showed good correlation to Gallery Total Polyphenol application performed according to the EBC 9.11 protocol. Recovery rates varied within 94 - 106 % and total precision (n=50) was 2.4 % or less for all samples. No significant run-to-run variation was noticed. Method linearity was determined in range of 79 - 199 mg/l but it can be extended with automated dilutions. No sample pretreatment was needed for the beer samples. Advantages of automation include easier and faster measurement. It also enables small sample sizes with less waste. For light sensitive reagents, the analyzer enables dispensing without reagent being exposed to excess light. In addition, with Gallery it is possible to perform simultaneously several other analytes for the same sample. For Total Polyphenol measurement the analysis turnaround time is approximately 10 minutes for one request and 55 minutes for 100 requests.

Introduction
Total polyphenols are becoming interesting group of compounds because of their beneficial, antioxidant effects in food. There are currently several analytical methods for the Total Polyphenol measurement. Folin –C based method and measurement at 280 nm are widely used methods in the wine industry but due to the unspicificity of these methods the result levels differs from the EBC method used for beer and wort samples. In the brewing industry, polyphenols are considered as beer fluoronoids such as the flavan-3-ols and their products, the proanthocyaninds, represent a class of readily oxidizable compounds capable of inhibiting or preventing the oxidation of other molecules present in beer (3); Total Polyphenols are analyzed due to their role in the chemical entities which control the colloidal stability of the beer, the other being proteins.

In this study, the EBC / MEBAK Total Polyphenol method (1,2) was adapted to Thermo Scientific Gallery discrete analyzer and automated method was optimized to 37 °C reaction temperature, which is the basic operation temperature in the Gallery analyzer. Results are reported in mg/l to the nearest whole number as Gallic acid equivalent. Seven BAPS reference beers from LGC Standards were used as samples in method comparison.

Materials and Methods

Instruments
Discrete photometric Thermo Scientific Gallery analyzer (manufactured by Thermo Fisher Scientific) is a fully automated bench-top system.

Samples
Seven BAPS reference beers from LGC Standards were used as samples in method comparison. Reference samples included both lager and bitter beers. Assigned values for each sample were determined as statistical median of results reported by participants.

Reagents
Reagents were prepared according to EBC protocol 9.11 with slight modification in Ammonia dilution ratio to avoid evaporation. However, the original reagent ratio was kept. Carboxymethyl cellulose/ ethylendiamine tetracetic acid (CMC/EDTA) was prepared by weighting 1.0 g sodium CMC and 0.2 g disodium EDTA and diluted to 100 ml with H.O. If necessary, the reagent can be clarified by centrifugation. Reagent is stable one month at 2-8 °C. Ferric reagent was prepared by weighting 3.1 g ammonium iron citrate with iron concentartion of 16 % and diluting it to 100 ml with H.O. Reagent is stable for one week at 2-8 °C if not exposed to light. It is recommended to store the reagent in a dark place in brown glass container or covered with foil. Ammonia reagent was prepared by diluting concentrated ammonia (d=0.92 g/ml) to deionized water with dilution ratio 1:39. All chemicals were purchased from Sigma-Aldrich.

Application
80 µl of CMC/EDTA reagent, 100 µl of sample and 65 µl of ammonia reagent were dispensed to the cuvette and blank was measured at 600 nm. 5 µl of ferric reagent was added and incubated in 420 s. The absorbance was measured at 600 nm. The Gallery analyzer computes sample concentration automatically using the formula:

\[ P = 820 \cdot (A - BIAS) \]

where P is the polyphenol content (mg/l) and A is the absorbance at 600 nm. The BIAS correction is done by measuring the response of deionized water.

Method Calibration
The value of the calibration type is set to ‘Bias’. The Bias calibration is measured with deionized water as calibrator by using one measurement point. The factor is set to 820 before measuring the bias of the calibration line.

Sample Pretreatment
No additional sample pretreatment was needed for beer samples. Turbid wort samples were centrifuged before the analysis.

Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Assigned value (mg/l)</th>
<th>Measured value (mg/l)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lager 1</td>
<td>159</td>
<td>159</td>
<td>99</td>
</tr>
<tr>
<td>Lager 2</td>
<td>75</td>
<td>74</td>
<td>94</td>
</tr>
<tr>
<td>Lager 3</td>
<td>110</td>
<td>106</td>
<td>98</td>
</tr>
<tr>
<td>Lager 4</td>
<td>135</td>
<td>136</td>
<td>101</td>
</tr>
<tr>
<td>Lager 5</td>
<td>102</td>
<td>101</td>
<td>98</td>
</tr>
<tr>
<td>Bitter 1</td>
<td>195</td>
<td>207</td>
<td>106</td>
</tr>
<tr>
<td>Bitter 2</td>
<td>142</td>
<td>133</td>
<td>94</td>
</tr>
</tbody>
</table>

Conclusion
The photometric method used in Thermo Scientific Gallery analyzer for analyzing Total Polyphenol in beer and wort correlates well with the EBC reference method. The advantage of using automated analyzer is the speed of analysis and the possibility to measure several analytes from the same sample in addition of Total Polyphenol measurement. This automated method can perform more than 100 Total Polyphenol measurements per hour with very little hands-on time.

Reference
2. Brautechnische Analysemethoden Band II, Methodensammlung der Mitteleuropäischen Brautechnischen Analysemethode (MEBAK).