



Total polyphenol content in ready to drink teas determined by Folin-Ciocalteu method and HPLC analysis

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Youngmok Kim, Ph.D. and Daniel J. Wampler, Ph.D.

ABSTRACT

Total polyphenol content in ready to drink (RTD) tea is generally determined by the Folin-Ciocalteu method. Since the Folin-Ciocalteu is not a specific method to measure only polyphenolic compounds, "total polyphenolic content" may not be the right term to describe the value obtained by the Folin's method. In the present study, total polyphenol content in RTD teas was determined by the Folin's method and HPLC analysis. As a result, total polyphenol content determined by the Folin's method was significantly higher than the value by HPLC analysis.

INTRODUCTION

RTD tea is a growing market segment based on convenience and perceived health benefits. Unlike other countries in Asia and Europe, about 80% of tea consumed in the US is served cold which is attributed to rapid market ascent in the US in relation to other countries (1, 2). Consumer shifts towards healthy beverages have also been a driving force for RTD tea in the US. Specifically, this market has grown to \$1.5 billion with 10% or more annual growth since early 2000's (2). Information is readily available on the chemical composition of green and black tea, but limited information exists on the phytochemical composition in RTD tea (3) or on the stability of these compounds during processing and storage. Since RTD teas are stored in solution for extended periods of time, most often without the aid or refrigerated distribution or storage, inherent changes during storage may occur which impact consumer acceptability and potential health benefits.

Many RTD teas claim their polyphenolic content but no information is available on what analytical method was used. There are two standard methods to determine total polyphenol content in tea drinks, the Folin-Ciocalteu method and HPLC analysis, both standardized by the International Organization of Standardization (ISO). In the present study, the differences between these two methods when determining total polyphenol content in RTD teas were evaluated.

MATERIALS AND METHODS

Material:

Twenty three (23) RTD teas were purchased from a local grocery market. Specific brand names are not mentioned in this research report.

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Analytical Methods:

1. Folin-Ciocalteu method: Total polyphenol content (also known as total soluble phenolics) was determined using Folin-Ciocalteu assay as described by Swain and Hillis (4). In brief, 300 μ L of each tea was added to test tubes and mixed with 1mL of 0.2N Folin's reagent. After 3 min of reaction, 1 mL of sodium carbonate solution was added to stop the reaction and to develop characteristic blue color for 7 min. Seven mL of purified water was added after that. Absorbance was measured at 726 nm and the total phenolic content was calculated based on slope from serial dilution of a gallic acid standard. The final value was expressed as gallic acid equivalent.
2. HPLC green tea analysis: Each green tea infusion was diluted 3-fold with reverse osmosis (RO) water and filtered through a 0.45 μ m PTFE syringe filter (Whatman, Clifton, NJ) prior to injection. Polyphenolic separations was conducted on a Agilent 1200 HPLC system using a Agilent G1315B Diode Array Detector (DAD) with a Dionex 250 x 4.6 mm Acclaim 120-C₁₈ column with a flow rate of 0.8 mL/min. A gradient mobile phase consisted of Phase A (100% H₂O) and Phase B (60% Methanol and 40% H₂O) each adjusted to pH 2.4 using *o*-phosphoric acid. The gradient started by running 0% Phase B for 1min, 0-30% Phase B over 30min, 30-80% Phase B in 15min, 80-100% Phase B in 15min, followed by a 5 minute equilibration time with 100% phase A for a total run time of 65min. Polyphenol compounds were detected and quantified at 280 nm against external standards of gallic acid, theobromine, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-epigallocatechin, (-)-epicatechin gallate, and caffeine, all procured from Chromadex (Chromadex Inc., Irvine, CA).
3. HPLC black tea analysis: Each black tea infusion was also properly diluted and injected to Agilent 1200 HPLC system with a ES industry FluoroSep-RP 150 X 4.6mm 5 μ m column with a flow rate of 1.0 mL/min. A gradient mobile phase consisted of Phase A (97% water, 2% Acetonitrile, 1% Methanol, and 0.1% phosphoric acid), Phase B (100% Acetonitrile), and Phase C (100% methanol) each adjusted to pH 2.4 using *o*-phosphoric acid. Total run time was 38min and polyphenol compounds were detected and quantified at 278 nm against external standards of theaflavin, theaflavin monogallate, and theaflavin digallate, all procured from Chromadex (Chromadex Inc., Irvine, CA).

RESULTS AND DISCUSSION

Overall, total polyphenol content determined by the Folin's method showed significantly higher quantity compared to the value by HPLC analysis (**Figure 1**). According to Brunswick laboratory, one of the largest antioxidant research facilities in the nation, the Folin's method is defined as "the Folin-Ciocalteu measures reducing capacity, which has normally been used to estimate phenolic contents of biological materials." Since the Folin's method detects not only polyphenolic compounds but also other biological compounds that have reducing power such as amino acids, carbohydrates, ascorbic acid, and saponin which are all naturally present in tea, the value by Folin's method might be higher than the value by HPLC. An addition of ascorbic acid to RTD teas and using coloring material such as caramel color also increases the value by Folin.

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Since those compounds (amino acids, ascorbic acid, carbohydrate, saponin, and caramel color) are not polyphenols, the value determined by the Folin's method could mislead health conscious tea drinkers.

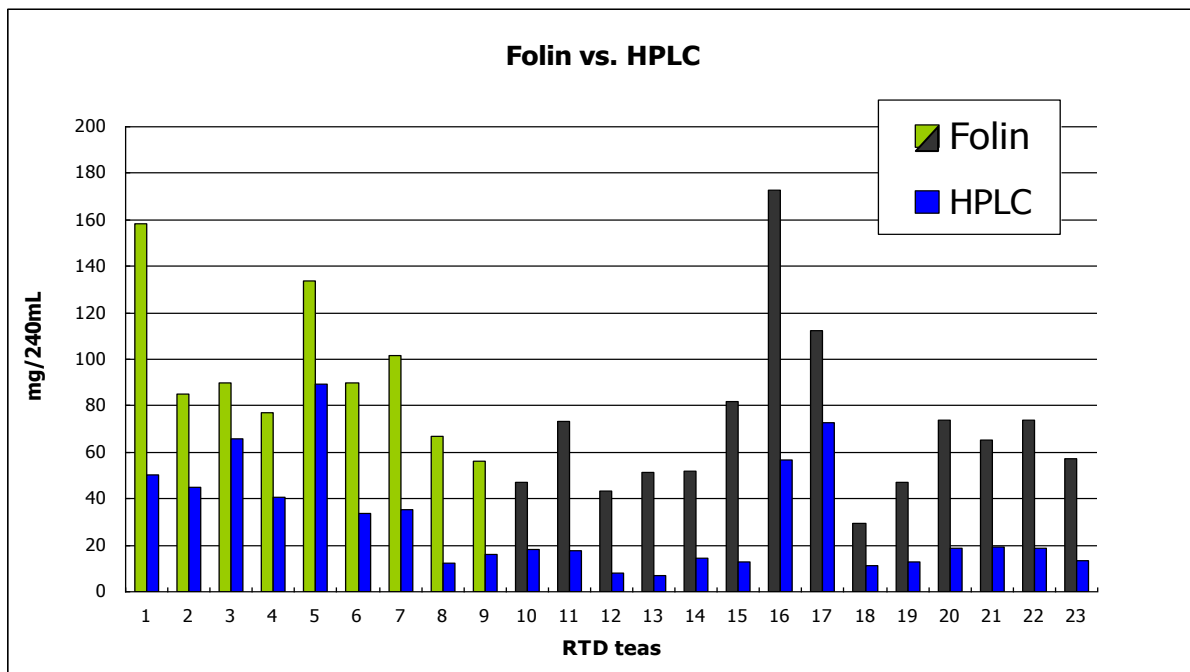


Figure 1. Total polyphenol content determined by Folin-Ciocalteu and HPLC analysis.

CONCLUSION

The two approved methods of determine polyphenolic content of RTD teas, the Folin-Ciocalteu chemical method and the HPLC instrumental method. The two methods are different, with different advantages and disadvantages. Using the Folin's method to determine total polyphenol content in RTD teas may mislead consumers because the true polyphenolic content is overstated.

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