

# **What influences total polyphenolic content in RTD teas when determined by Folin-Ciocalteu method**

Sensus Technical Note (SEN-TN-0033)

09/07/2011

**Youngmok Kim, Ph.D.; Kevin L. Goodner, Ph.D.**

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## **ABSTRACT**

In the present study, the factors that could influence the total polyphenol content in ready to drink teas were investigated when determined by Folin-Ciocalteu method. Ascorbic acid and caramel color addition increased total polyphenol content of RTD teas even though they are not polyphenolic compounds.

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## **INTRODUCTION**

The Folin-Ciocalteu method was first developed in 1959 to determine protein content and later modified and optimized to determine total polyphenol level in 1977. This method is officially approved by the US Tea Association and also standardized by ISO in 2005 for tea polyphenol determination. So, the Folin's method is generally considered as the standard method for tea polyphenol determination. This method is defined by Brunswick laboratory, which one of the largest antioxidant research groups in the nation as "the Folin-Ciocalteu measures reducing capacity, which has normally been used to estimate phenolic contents of biological materials".

Polyphenol compounds have reducing capacity, but the problem is that polyphenols are not the only compounds that have reducing capacity which naturally present in tea. Besides polyphenols, some other compounds present in tea such as protein, carbohydrate, vitamin C, and saponin, which all have reducing capacity and their presence is also detected by the Folin's method.

In this study, increase in total polyphenolic content determined by Folin's method in RTD tea beverages is determined.

## **MATERIALS AND METHODS**

### **Materials:**

1. Reverse Osmosis (RO) water
2. Ascorbic acid, sodium carbonate, and Folin's reagent from Sigma Aldrich, St. Louis, MO
3. Food grade caramel color

### **Sample preparation:**

1. Ascorbic acid solution was made from 0mg/240mL to 200mg/240mL using RO water.
2. Caramel color solution was prepared from 250mg/L to 400mg/L using RO water.

**Methods:**

Total polyphenolic content (also known as total soluble Phenolics) was determined using Folin-Ciocalteu assay as early described by Swain and Hillis (1959). In brief, 300 µL 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. Known amount 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 gallic acid standard. The final value was expressed as gallic acid equivalent.

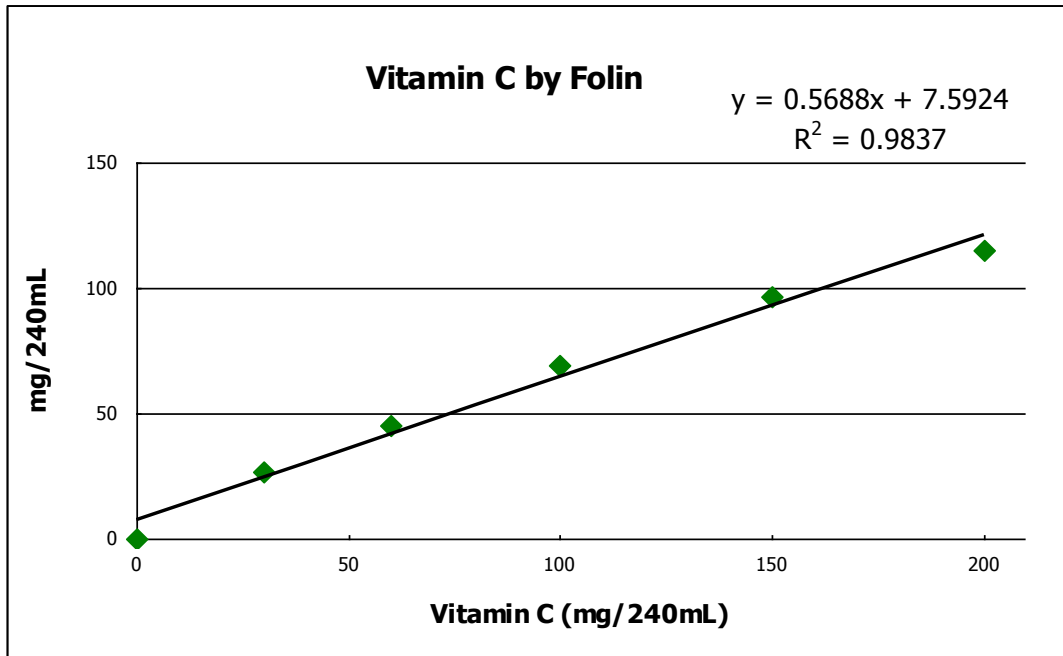
**RESULTS AND DISCUSSION**

Some tea beverages claim vitamin C content in their beverages. Ascorbic acid is a popular food additive because it increases storage stability of food. Also, they can improve the nutritional value of products meaning they can claim vitamin C in their products. Several dilutions with vitamin C were made and the solutions were tested with the Folin’s method. According to the data obtained in this study, ascorbic acid addition significantly increased total polyphenol content even though it is not a polyphenolic compound (**Table 1, Figure 1**).

**Table 1.** Total phenolic content determined by Folin-Ciocalteu assay after ascorbic acid addition to Sensus black tea. Values were expressed as gallic acid equivalent.

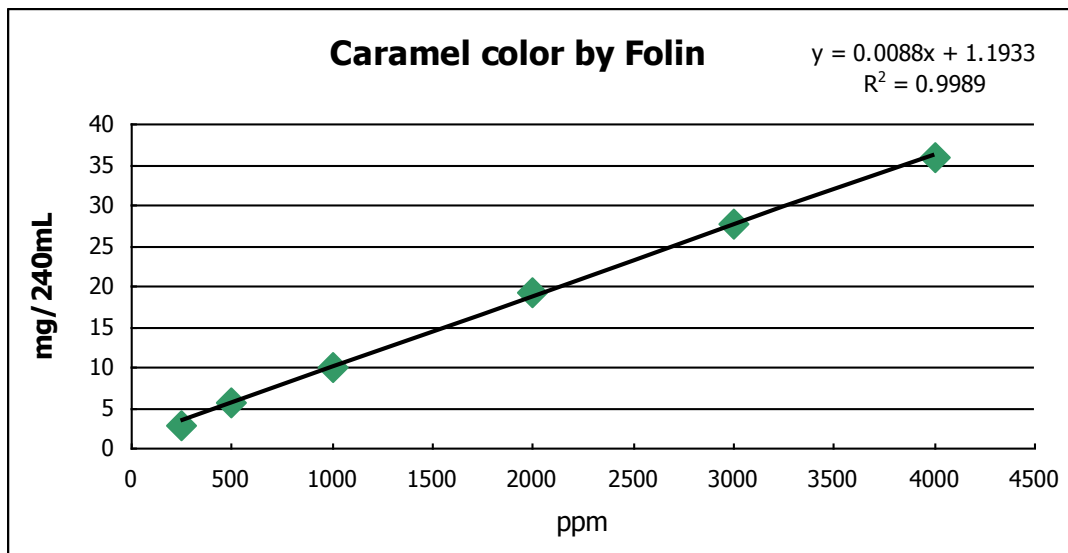
| The amount of added ascorbic acid   | 0mg/8 oz       | 30mg/8 oz | 60mg/8 oz | 100mg/8 oz | 150mg/8 oz | 200mg/8 oz |
|-------------------------------------|----------------|-----------|-----------|------------|------------|------------|
| Total polyphenol content (mg/240mL) | 0 <sup>1</sup> | 26.87     | 45.30     | 69.02      | 96.66      | 114.87     |

**Figure 1.** Total polyphenol content in ascorbic acid solution by Folin-Ciocalteu method. Values were expressed as gallic acid equivalent.

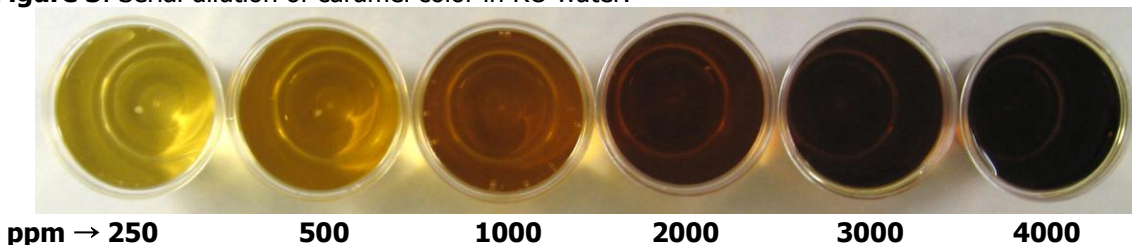


Caramel color is sometimes added to tea beverages to mimic tea color. Several dilutions of a caramel color were made and the Folin's test was conducted. The amount of caramel color to match a commercial black tea color was 4000ppm and it resulted in 37 mg per serving of polyphenols measured by the Folin's method (**Figure 2, Figure 3**).

**Figure 2.** Total polyphenol content in caramel colored water determined by Folin's method. Values were expressed as gallic acid equivalent.



**Figure 3.** Serial dilution of caramel color in RO water.



## **CONCLUSION**

Folin's method is an official method adopted by US tea association. They say that Folin's method can be used for all kinds of tea including freshly brewed and ready to drink teas. However, RTD teas often have other ingredients such as ascorbic acid and caramel color which can affect the Folin's value. Therefore, it is critical to keep this in mind that addition of ascorbic acid and caramel color can inflate total phenolic content when applying the Folin's method to ready to drink teas.

## **REFERENCE CITED**

Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Food. Agric.* **1959**, *10*, 63-68.