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Quantification and Scavenging Ability of Antioxidants in Bottled Tea

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Antioxidants in bottled tea: activity and quantification

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Introduction

• Bottled tea is made from brewed leaves of the *Camelia sinensis* plant. Different fermentation processes of the leaves lead to different types of tea, including black, oolong, green, and white tea.

• Tea leaves contain molecules called polyphenols that have aromatic rings with many alcohol groups. The polyphenols present in tea have antioxidant properties, which have been seen to prevent certain types of diseases[2].

• Antioxidants work by stabilizing highly unstable free radicals, which are missing an electron in their orbital. Normal cellular processes, such as peroxisome detoxification, generate free radicals. Environmental pollutants such as cigarette smoke and pesticides can also produce them. The unpaired electron in a reactive oxygen species decreases its stability, allowing the species to react with different structures within the system, such as cell membranes. Reactive oxygen species cause harmful damage that is often irreversible, and if not repaired can lead to cell death or accumulations of mutations that can cause cancer. Antioxidants donate an electron to the reactive oxygen species, thus losing one electron out of its cloud and completing the electron pair in the reactive oxygen species[7].

•Different studies have concluded contradictory results about which type of tea contains the highest quantity of antioxidants. The goal of our research is to analyze the quantity of different antioxidants present in bottled tea, and then analyze their quality and activity of effectiveness[6].

Objective 1: Use colorimetric assays to quantify the content of polyphenols in bottled tea and compare our findings to the same tests on brewed tea.

Objective 2: Analyze the effect of additives (such as sweeteners, milk products, and flavorings) on the amount and quality of antioxidants in tea.

Objective 3: Study the effectiveness of antioxidants in bottled tea using direct methods: like cyclic voltammetry and indirect methods like radical scavenging with ABTS.

References

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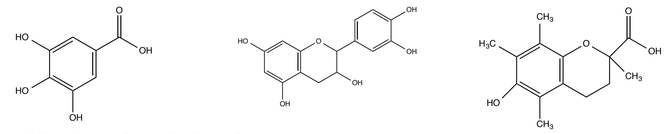
Methods

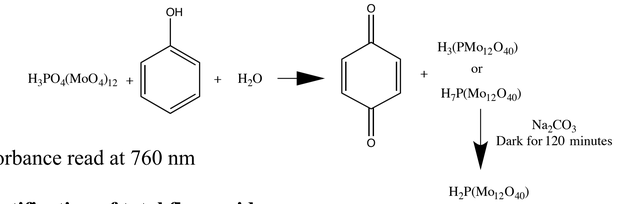
Measurements of colorimetric Assays taken using Cary 300 UV-Visible Spectrophotometer[2]. All teas purchased from Kroger Grocery store, Bloomington, IL. Bottled teas included Gold Peak (Green Tea with Honey), Arizona Iced Tea (Green Tea with Ginseng and Honey, and Sweet Black), and Pure Leaf Tea (Sweet Black, and Unsweetened Black).

Standards

Gallic Acid (for phenols) Catechin (for flavonoids) Trolox



Quantification of total phenols



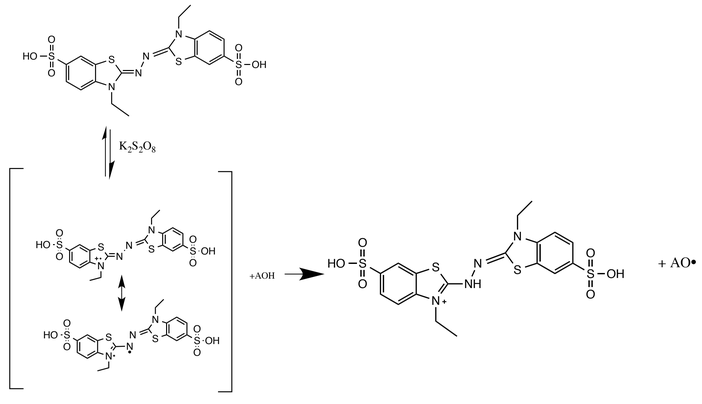
Absorbance read at 760 nm

Quantification of total flavonoids

Solution of 5% $NaNO_2$, 10% $AlCl_3$, ultrapure water , and bottled tea prepared and left in dark for 10 minutes at room temperature. Absorbance measured at 415 nm of the complex made with flavonoids in tea and $AlCl_3$ (forms a brownish red color).

ABTS Assay

Solution of 6.3 mM ABTS (2,2'-azinobis-93-ethylbenzothiazoline-6-sulfonic acid), 2.45 mM $K_2S_2O_8$, and tea dilution prepared and left in dark for 2 hours. Absorbance is read at 760 nm.



Quantification of theaflavins (TF) and thearubigins (TR)

Typically theaflavins are quantized using a well-known Flavognost method. Given that bottled tea has a much lower concentration than brewed tea, this method was unsuccessful. Therefore, we are using another separation method to study TR quantitatively.

Sample: tea extract prepared of 1% (w/v) Na_2HPO_3, Tea, and Ethyl Acetate.
Reference samples (R_1= Ethyl Acetate and Methanol; R_2= 10% (w/v) oxalic acid, water, and methanol).
For TF, measured at 380 nm with sample of tea, ethyl acetate extract and methanol.

Percentage = absorbance*2.23

For TR, absorbance read at 460 nm with sample of tea, 10% (w/v) oxalic acid, water, and methanol.

Percentage = $7.06*(4E_2-E_1)$

Results

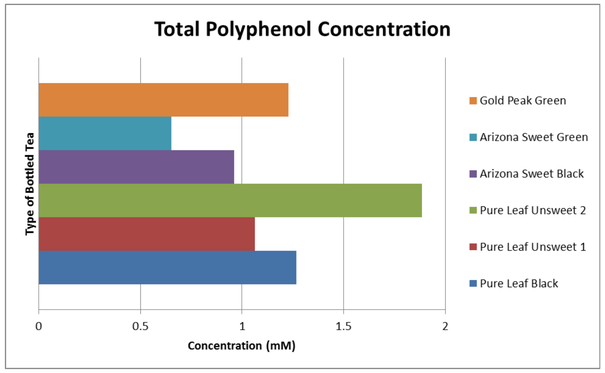


Figure 1.1 Quantification of total phenol concentration in bottled tea. Range of concentrations from 0.652 mM (Arizona Sweet Green) to 1.89 mM (Pure Leaf Unsweetened 2). For brewed tea, the range of concentrations was 1.47 mM (Souchong black tea) to 6.49 mM (white tea).

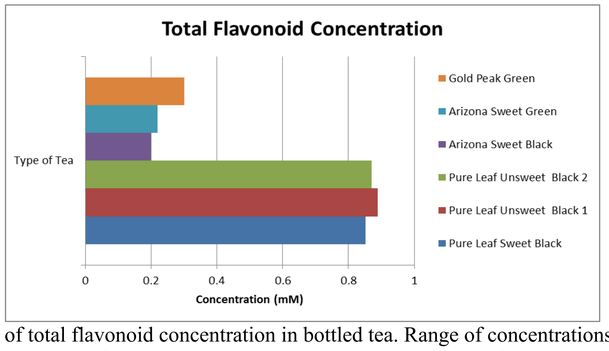


Figure 1.2 Quantification of total flavonoid concentration in bottled tea. Range of concentrations from 0.200 mM (Arizona Sweet Black) to 0.890 mM (Pure Leaf Unsweetened 1). For brewed tea, the range of concentrations was from 2.709 mM (Lapsong black tea) to 5.931 mM (white tea).

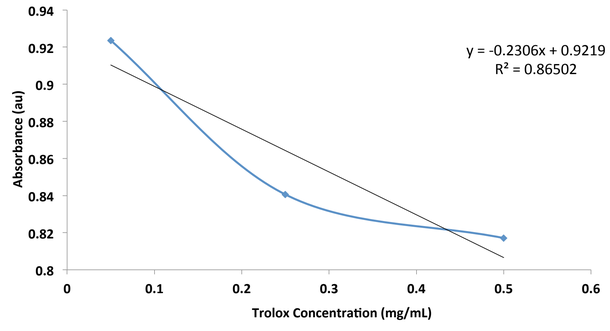


Figure 1.3 Trolox Standard Calibration Curve. The range of absorbance values for bottled teas of various concentrations with ABTS was -0.549 au to -0.133 au, which extends beyond the range of the calibration curve, indicating that this protocol must be optimized.

Table 1. Mass of theaflavins and thearubigins in 0.5 grams of brewed tea.

	White	Black
TF	0.0071 g	0.0063 g
TR	0.01512 g	0.1468 g

Conclusions

Upon preliminary analysis, it appears that bottled tea contains a lower quantity of polyphenols, compared to both the literature and the data that has been collected on brewed tea at Illinois Wesleyan University. Our data suggests that bottled unsweetened black tea has the highest amount of polyphenols as well as flavonoids.