Comparison of Polyphenols and Fluoride content in Commercial Brands of Organic and Non-organic Green Tea

by

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Abstract

Growing consumer demand for organic products has been manifested in the marketplace in many ways. If an organic food product has functional properties that lead to improved health, it will have added value compared to regular products. Organic green tea falls into that category due to its rich polyphenol content. However, the tea plant accumulates and stores fluoride at higher levels than most other plants. Neither non-organic nor organic varieties of tea plants can escape fluoride contamination resulting from water, soil and the air pollution during the cultivation. In this study polyphenol and fluoride contents were determined in non-organically and organically grown green teas. Polyphenols were determined by HPLC. Fluoride was determined using an ion selective electrode. Results showed that total polyphenol content in non-organic green tea ranged from 35.1-88.8 mg/g of tea compared to organic green tea, 54.6-91

mg/g, and fluoride content in organic and non-organic green tea ranged from 0.08-0.47 mg F^-/g tea leaves 0.10-0.37 mg F^-/g tea leaves respectively.

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Chapter I: Introduction

Tea is a beverage made by steeping processed leaves from the plant, *Camellia sinensis* in hot water (Rasmussen & Rhinehart, 1999). Next to water, tea is the most widely consumed beverage in the world (Tea association of the USA, 2001). There are four general types of tea; oolong, black, white and green tea (Imperial tea garden, 2003)

All types of tea contain polyphenolic compounds at various levels (Dubick & Omaye, 2007). As natural antioxidants (Koketsu, 1997), a number of health related functional properties have been attributed to polyphenols in tea. Among them are anticarcinogenic (Kim & Masuda, 1997), antimicrobial (Matsumoto, Okubo & Akachi, 1997) properties and the ability to prevent dental caries (Sakanaka, 1997). Of the four types, green tea is richest in polyphenols, because polyphenol oxidase is rapidly inactivated by steaming, rapid pan firing, rolling and high temperature air drying during processing. This has generated interest in the importance of green tea relative to human health (Dubick & Omaye, 2007).

Organic foods have been a rapidly growing sector in the food industry over the past decade. In the United States, organic food sales are rising at a rate of more than 20 percent annually (Baourakis, 2004). There are several reasons for this trend; the most important is health related factors. Organic foods are pesticide and chemical free.

While tea might have beneficial effects against cancer, diabetes and pathogenic bacteria (Friedman et al., 2005), tea leaves can accumulate larger amounts of fluoride than other edible plants (Schuld, 1999). Increased fluoride levels can result from a variety of conditions including increased fluoride levels in the soil, air, water and chemical fertilizers used during cultivation.

Statement of the problem

Green teas have more polyphenols than other types of tea (Matsumoto, Okubo & Akachi, 1997). However, very little research has been reported on the effects of organic farming on polyphenol content in green tea. This research was designed to compare polyphenol content in commercial green tea from organic and non-organic sources. In addition, given that tea plants can concentrate fluoride, it was also the intent of this project to determine fluoride levels in both organic and non-organic green tea products.

Objectives of the study

The objectives of this research are

1. quantification of polyphenols in organic and non-organic green tea and
2. quantification of fluoride content in the organic and non-organic green tea.

Definition of terms

Antioxidant. A substance that limits oxidation or inhibits reactions promoted by oxygen or inhibits reactions promoted by oxygen or peroxides, many of these substances being used as preservatives in various products.

Organic foods. Foods that are produced without using artificial pesticides or fertilizers or sewage sludge; bioengineering; or irradiation in accordance to the regulations in USDA.

Green tea. Tea made from the two leaves and a bud of *Camellia sinensis*. Leaves are steamed, rolled and dried without fermentation.

White tea. Tea made from the young leaves or buds of *Camellia sinensis*. Leaves are steamed and dried without rolling and fermentation.

Polyphenols. Chemical compounds containing at least two benzene rings with one or more alcohol groups (-OH) attached to each ring. Many plants, like tea, fruits and vegetables, contain natural polyphenols such as flavonoids and tannins that are known for their health related benefits.

Assumptions of the Study

All tea samples used in this study were made from the plant *Camellia Sinensis*. None of the teas had intentional post harvest alterations of polyphenol or fluoride content. Teas were similar in post-harvest aging and handling. Teas labeled "organic" were grown and manufactured according to accepted organic practices.

Chapter II: Literature Review

Tea is said to have been discovered by Shen nung, a Chinese emperor, who ruled from 2737 to 2697 B.C. (Campbell, 1995). In another story, a Buddhist saint traveled to China to spread Buddhism from his native India. He had made a promise to spend nine years in meditation. During his fifth year he suffered extreme fatigue. He then discovered the tea plant and made an infused beverage from its leaves. After he drank the beverage he was revitalized and successfully completed his meditation (Rasmussen & Rhinehart, 1999; Campbell, 1995). While it is generally accepted that the tea trade started in China, both China and India claim to have identified the nutritional properties of tea (McNulty, 2004).

About Tea plant

Botanically all the tea plants belong to the *Camellia* family. Scientists have divided tea plants into two species, the Chinese plant (*Camellia sinensis*) and the Assam plant (*Camellia assamica*) (Donhauser, 2006). As seen in Figure 1, tea leaves are dark green and the blossom is cream colored and aromatic. Only the top two leaves and a bud are used in tea processing. Tea plants can grow to a maximum height of about 30 feet, but under cultivation they are pruned to 3-5 feet to maximize leaf production and facilitate harvesting (Tea, 2011).

Figure 1: Tea plant with two leaves and a bud on the top along with blossoms (Botanical Tea Plant, 2010).

Types of tea

Based on the method of processing, teas are classified into four types: black, oolong, green and white tea. The different processing steps for each type of tea are shown in the Figure 2.

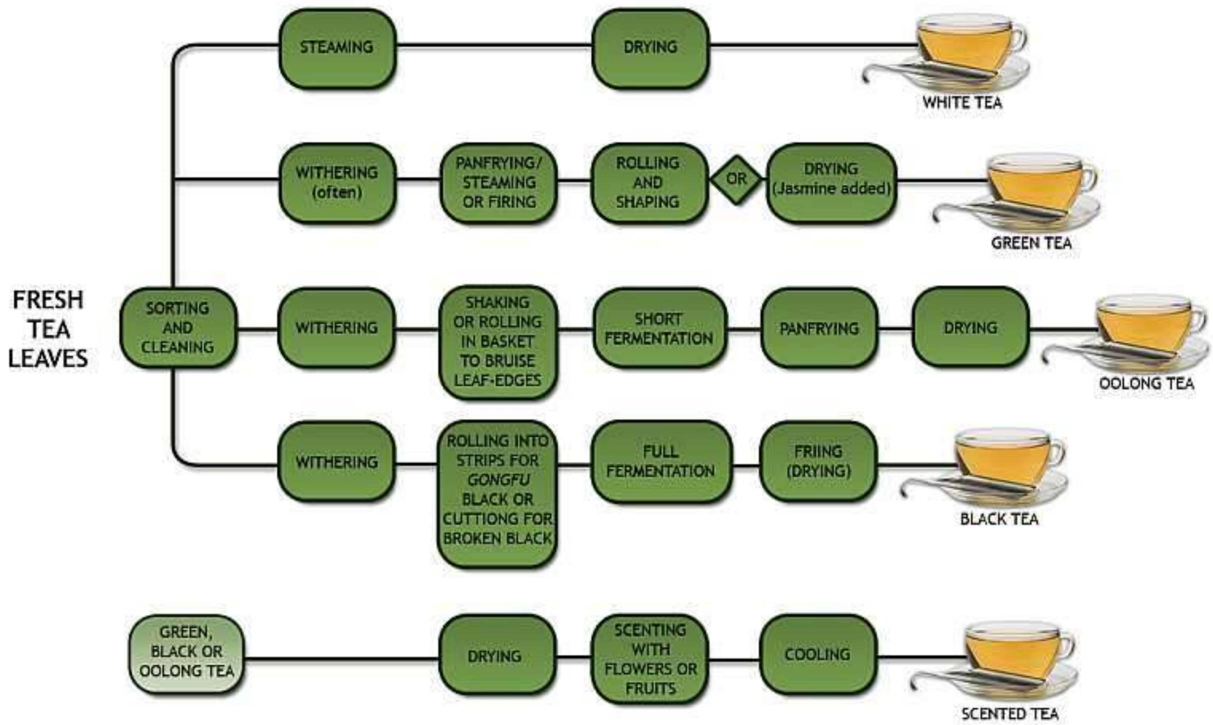

Figure 2: Processing steps in different types of tea (Source: Tea Processing, 2005).

For manufacturing green, oolong and black teas, two leaves and a bud are used whereas for white tea, buds or young leaves are used. Below are the steps used for manufacturing green tea.

Withering

Withering reduces the moisture content of tea leaves. Leaves are spread on trays or fine meshed screens, and hot air is passed through them for 12-17 hours (Tea production process, 2001). Air temperature is 80-90^{o}F and relative humidity is 70-80% (Ukers, 1935). At the end of withering, the final moisture is reduced to 70%, and tea leaves are flexible enough to be rolled (Tea production process, 2001). The withering step can be omitted for green tea, but it is always used to process black tea (Green tea, 2003).

Steaming

Tea leaves are steamed at 95-100^{o}C for about 30-45 seconds to inactivate the polyphenol oxidase enzyme thus preventing the loss of polyphenols through oxidation. Steaming also helps prevent loss of vitamins and is the reason why green teas have higher vitamin content than the fermented teas (Chu, 1997).

Rolling

The objective of rolling is to break up the leaf cells so that essential oils are released, giving particular teas their unique aroma. Rolling also gives a particular shape and distinguished twist to the tea leaves. During rolling the temperature of the leaves must be kept below 85^{o}F to prevent fermentation and damage to the final product (Ukers, 1935).

Drying

Rolled tea leaves are dried immediately using hot air dryers to reduce moisture content to 3% and stop the oxidation process. Dried tea leaves are ready for packing and distribution (Tea production process, 2001).

Chemical composition of tea

Cellulosic carbohydrates are the largest constituents in tea followed by protein and lipids. Because they are insoluble, they do not become part of the tea beverage, Only small molecular weight components like polyphenols, caffeine, theobromine, vitamin C, magnesium, aluminum and fluoride diffuse into tea water (Chu & Juneja, 1997).

Tea polyphenols are maximally diffused into boiling water. The slight astringent and bitter taste of the tea infusion is due to polyphenols. The tea polyphenols are composed of six catechins and their derivatives. Individual polyphenol content deviates slightly depending upon species and time of harvesting. Epigallocatechin gallate (EGCG) is the polyphenol present in the greatest amount followed by Epigallocatechin (EGC), Epicatechin gallate (ECG), and Epicatechin (EC).   Catechin gallate (GC) and Catechin (C) are present in trace amounts compared to others (Chu & Juneja 1997).   The chemical structures of these polyphenols are shown in Figure 3.

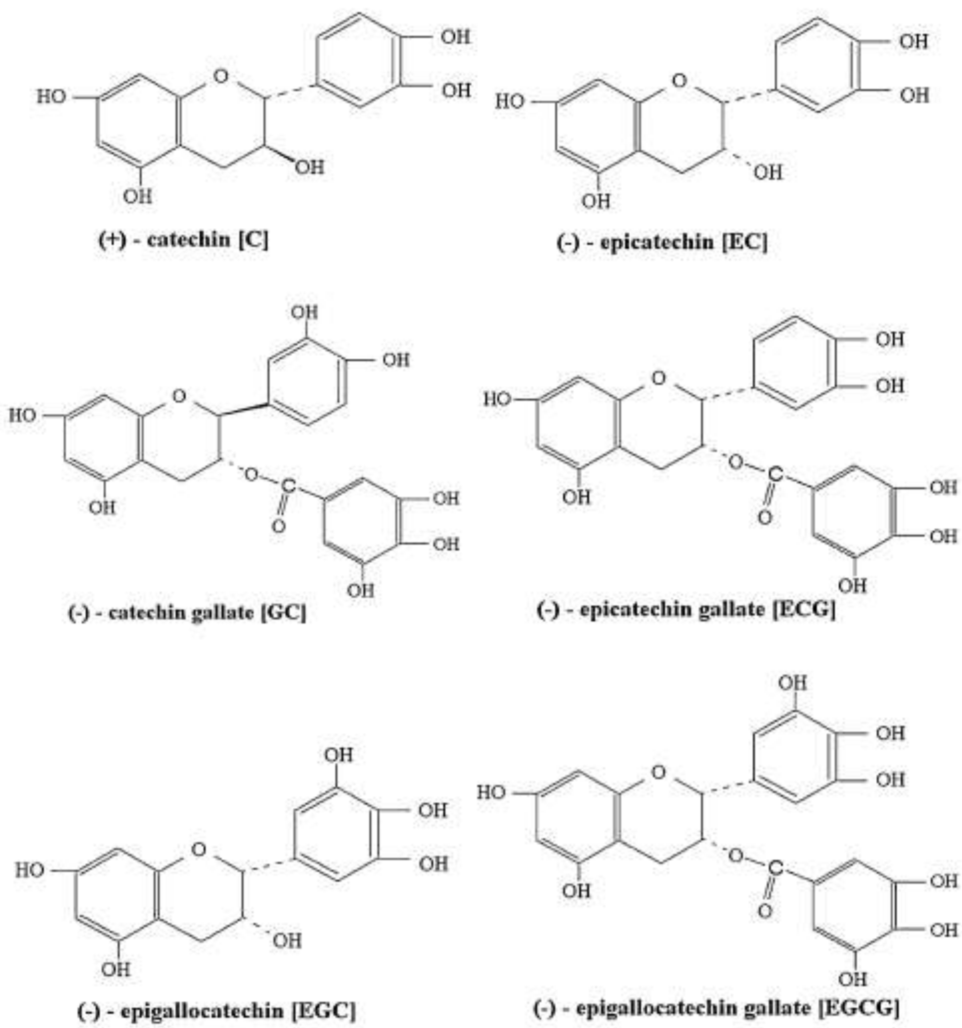

Figure 3: Chemical structures of the tea polyphenols (Gramza, & Korczak, 2005)

Polyphenols in green tea

Kafley (2008), used reversed phase HPLC to quantify the polyphenol content of commercial green and decaffeinated green teas using reverse phase HPLC. Total polyphenol content ranged from 52.4-239.0 mg/serving in green tea and 88.0-143.0 mg/serving in decaffeinated green tea. Table 1 shows the total polyphenol content of green and decaffeinated green teas in mg/serving tea. Kafley found that in all the brands analyzed, green tea had more total polyphenol per serving than its decaffeinated counterpart except for Bigelow brand where the decaffeinated green tea had the highest polyphenol content.

Table 1

Total polyphenol content of green and decaffeinated green teas in mg/serving tea

Brand	Green tea	Decaffeinated green tea
Bigelow	52.4±1.8	112.0±4.0
Celestial seasoning	143.0±15.0	88.0±15.0
Lipton	180.0±20.0	143.0±17.0
Our family	139.0±19.0	116.0±7.0
Red rose	239.0±20.0	132.0±9.0
Salada	202.0±18.0	105.0±21.0
Stash	227.0±13.0	140.0±18.0

(Source: Kafley, 2008)

Gudala (2008) reported the effect of temperature and time on the extraction of total polyphenols in commercial green and decaffeinated green teas. In all the teas, total polyphenol content increased as brewing temperature increased. When was measured as a function of brewing temperature and time, total polyphenol content in tea increased to a maximum at 7 hours. The author suggested that the decrease in total polyphenol content at longer brewing times may be due to the oxidation of polyphenols. Overall, extraction of total polyphenol content reached a maximum when brewed for 10 minutes at 100^{o}C. Table 2, shows the highest total polyphenol content found in three tea brands.

Table 2

Total polyphenol content in green tea

Brand	Type	Total polyphenol content mg/serving tea
Bigelow	Caffeinated	109.0
	Decaffeinated	194.0
Celestial Seasonings	Caffeinated	280.0
	Decaffeinated	177.0
Salada	Caffeinated	239.0
	Decaffeinated	147.0

(Source: Gudala, 2008)

Friedman et al. (2005) developed an HPLC method to estimate total polyphenols in 77 commercially available black, green, herbal, and specialty (brown rice, white and oolong) teas. They found that brewing time from 3 min to 20 min had no significant effect on total polyphenols measured. Organic Darjeeling green tea had the highest polyphenol content at 100.0 mg/g tea leaves, and organic spice herb tea had the lowest polyphenol content at 1.2 mg/g tea leaves. Total polyphenol content ranged from 6.5-72.5 mg/g black tea leaves, 4.4-100.0 mg/g green tea leaves,1.2-26.4mg/g herbal tea leaves, and 3.4-83.1 mg/g specialty tea leaves. The variation in polyphenol content in commercial tea is due to age, climate, agricultural practices, post harvest processing, and packaging.

Henning et al. (2003) measured polyphenol content in green tea by brewing tea leaves in 100 ml boiling, deionized water for 3 minutes. Brewed tea was filtered through a coffee filter and the filtrate was analyzed for polyphenols by high performance liquid chromatography. Green tea had 59.3-103.2 mg polyphenol/g tea leaves . Decaffeinated green tea contained 26.7-52.5 mg polyphenol/g tea. Black tea contained 21.2-68.3 mg polyphenol/g tea leaves and decaffeinated black teas had 4.6-5.4 mg/g tea leaves. Black tea has less polyphenol than green tea because fermentation of black tea lowers polyphenol content by generating epicatechin polypmers such as theaflavins, thearubigins and their gallate derivatives Henning et al. (2003).

Cabrera, Gimenez & Lopez (2003) measured polyphenols in a set of 45 samples of green, oolong, red and black teas commercially available in Spain. All the samples were in loose tea formats. The samples were prepared by extracting 2.5 g minced and ground tea three times in 20 ml of 80% (v/v) methanol for 3 hours and then two times in 20 ml of 80% (v/v) methanol, 0.15% (v/v) HCl for 3 hours. The combined extract was filtered through cotton followed by 0.45 µm filter paper to remove any particulates before injecting into the HPLC. EGCG, EGC, ECG and EC were the individual polyphenols measured. The total polyphenol content was highest in green tea (126.0-201.3 mg/g tea leaves) and lowest in oolong tea (25.7 mg/g tea leaves). Polyphenol in red and black teas were also very low (8.0-19.4 mg/g tea leaves, and 40.8-123.0 mg/g tea leaves respectively).

Longo et al. (2008) used reverse phase HPLC to measure EGCG and ECG in 25 major Indian cultivars. EGCG varied from 1.36-68.35 mg/g tea leaves, and ECG varied from 0.32-27.39 mg/g tea leaves.

Health benefits of antioxidants in tea

Tea polyphenols have several purported health benefits. Green tea polyphenols have been associated with claims to protect against allergies, tooth decay, high blood pressure and free radical formation (An et al., 2004). They may be effective against certain cancers (Mande, Weinreb, Amit, & Youdim, 2004; Michels, Willett, Fuschs, & Giovannucci, 2005), cerebral damage (Suzuki, Tabuchi, Ikeda, Umegaki, & Tomita, 2004), and diabetes (Vinson, & Zhang, 2005).

Organic foods

"Organic refers to the way agricultural products are grown and processed. It includes a system of production, processing, distribution and sales that assures consumers that the products maintain the organic integrity that begins on the farm" (Organic agriculture and production, 2003). Demand for organic products worldwide remains strong with sales growing by over $5.0 billion per year. It is estimated that global sales of organic foods reached $46.1 billion in 2007 (Willer & Klicher, 2009). Growth of organic food sales in the United States increased by almost 20% annually since 1990 (Winter & Davis, 2006), with a total trade output of $16.9 billion in 2006 (Natural and Organic Foods, 2007).

Consumer awareness of the relationship between foods and wellness, along with environmental incentives, has led to an increased demand for organic foods (Chassy, Bui, Renaud, Horn, & Mitchell, 2006). In a recent survey, Whole Foods Market (2005) found that 70.3% of consumers buy organic foods to avoid pesticides, 68.3% for freshness, 67.1% for healthy and nutritious alternatives, and 55% to avoid genetically modified foods. Consumer confidence in conventional produce has dropped because of food incidents involving mad cow disease, foot and mouth disease, pesticides, antibiotics, and other chemicals used to grow and process food (Siderer, Maquet, & Anklam, 2005).

Use of fertilizers and pesticides may limit the synthesis of components such as phenolic metabolites in the plants (Winter & Davis, 2006). Asami, Hong, Barrett and Mitchell (2003) reported that total phenolics in freeze and air dried marionberries and corn were significantly higher in organically grown produce compared to those grown conventionally. Carbonaro, Mattera, Nicoli, Bergamo and Cappelloni (2002) found that organic peaches and pears had significantly higher total polyphenols than their non-organic counterparts. Similarly Caris-Veyrat et al. (2004) found that organic tomatoes had higher levels of polyphenols, vitamin C and carotenoids than conventionally grown tomotoes. However, Young, Zhao, Carey, Welti, Yang and Wang (2005) found no difference in phenolic levels of organic and conventionally grown lettuce and collards. Mikkanon et al. (2001) found no difference in flavonol levels of organic and conventionally grown black currants. Plants respond differently to organic and non-organic horticulture practices.

Fluoride

Fluoride (F^-) is the anionic form of fluorine, a halogen and the most electronegative of all the elements in the periodic table. It is omnipresent in nature, and most sources of water contain at least small quantities of fluoride. The quantity of fluoride present in water is highly reliant upon geography. Generally, water has a fluoride content of approximately 2.0 ppm. In the areas with epidemic levels of skeletol and/or teeth fluorosis, fluoride contents in water can range from 3.0-20.0 ppm (Liteplo, Gomes, Howe, & Malcolm 2002). Fluoride is an essential trace element for humans, but either deficit or excessive fluoride intake can result in health issues. Fluoride at 1 mg/L in water prevents tooth decay, but at 5mg/L causes dental and skeletal fluorosis. Table 3 shows the recommended dietary intake of fluoride for different age groups (B. Li, S. Li, Wang, Liu, & Zheng, 2008).

Table 3

Recommended adequate intake of fluoride for different age groups

Age group	mg/day
0-6 months	0.01
7-12 months	0.50
1-3 years	0.70
4-8 years	1.00
9-13 years	2.00
14-18 years	3.00
>=19 years (males)	4.00
>=19 years (females)	3.00

(Source: United States Department of Agriculture, 2011)

Fluoride in food and beverages

Almost all the food products contain at least small amounts of fluoride. Higher levels are present in fish and tea leaves (Liteplo, et al., 2002). Table 4 shows the fluoride concentration in representative food products. Tea plants accumulate and store fluoride at higher levels than most other plants (Zerabruk, Chandravanshi, & Zewge, 2010). Most of the fluoride in tea plants is stored in the leaves and concentration increases with age of the leaves (Wong, Fung, & Carr, 2003). As a result, teas can be rich in fluoride (Zerabruk et al., 2010).

Table 4

Levels of fluoride in representative food products

Food	Fluoride concentration (mg/kg)
Milk and milk products	0.01-0.051
Meat and poultry	0.01-1.7
Fish	0.21-4.57
Soups	0.41-0.94
Baked goods	0.04-1.85
Vegetables	0.01-1.34
Fruits and fruit juices	0.01-2.8
Fats and oils	0.05-0.13
Sugars and candies	0.01-0.31
Beverages	0.003-1.28
Tea	0.005-371

(Source: Liteplo et al., 2002)

Zerabruk et al., (2010) used an ion selective electrode to measure the fluoride content at three different brewing times in 21 commercial Ethiopian and imported black and green teas. They found that as brewing time increased fluoride content of the tea increased. Fluoride ranged from 80-634 mg/(kg tea), 111-682 mg/(kg tea) and 130-728 mg/(kg tea) at 3 min, 5 min and 10 min brewing times respectively. Fluoride content in green teas, black tea leaves and black tea bags brewed for 5 minutes ranged from 111-190 mg/(kg tea), 117-683 mg/(kg tea) and 141-246 mg/(kg tea) respectively.

Shyu, Chen and Lee (2009) found that the fluoride content in twelve commercially available teas ranged from 100-451 mg/kg dry weight of tea leaves. In 1% and 2% tea leaves brewed for 5 minutes, fluoride content ranged from 0.39-1.21 mg F^{-}/L to 0.65-2.53 mg F^{-}/L.

This study was designed to quantify fluoride and polyphenol content in commercial brands of organic green tea, non-organic green tea and white tea, and to determine if selection of commercial green tea on the basis of its organic or non-organic production is a reliable predictor of polyphenol content.

Chapter III: Methodology

Introduction

Quantification of polyphenols in commercial green tea samples was accomplished by extracting water soluble compounds in tea using hot water followed by reverse phase, high performance liquid chromatography (HPLC). Fluoride content in the tea extract was measured using a fluoride ion selective electrode.

Sample selection

Organic and non-organic green teas were purchased from the Vitamin Shoppe in Edison, NJ. To avoid any interference from flavor compounds, flavored tea samples were not selected. The samples selected included organic green tea, organic white tea and non-organic green tea. These samples were packaged in tea bags except for the white tea, which was sold in loose-leaf format. Recognizing that tea products had different ages and shelf lives, and that specific leaf selection and processes could differ, products selected were representative of green and white, organic and non-organic teas available in the commercial marketplace. The type and brand of teas used is listed in Table 5.

Table 5

List of samples used in this study

	Brand of Tea	Type	g/tea bag
Organic Green Tea	Dancing	Tea bag	2.0
	Revolution	Tea bag	2.2
	China	Tea bag	1.8
	Tulsi	Tea bag	1.8
Non-organic Green Tea	Lipton	Tea bag	1.6
	Yogi	Tea bag	1.9
	Tropical	Tea bag	2.2
	White (organic)	Loose tea	-

Sample preparation

Following the label recommendations one tea bag, 2 g tea in the case of white tea, from each sample was placed into 250 ml of boiling distilled/deionized (Milli-Q) water for 5 minutes. Upon removal, each bag was squeezed to maximize extraction of polyphenols and fluoride. Two ml of the tea extract was filtered through 0.45 µ filter paper into an HPLC vial. The remaining tea extract was saved for fluoride analysis.

Standards preparation

Catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), and Epigallo catechin (EGC) were used as standards in determining individual polyphenol concentration from HPLC sample elution profiles. Twenty-five mg of standard was weighed into a 25 ml volumetric flask and diluted to 25 ml with methanol. The concentrations of the serial dilutions are listed in Table 6.

Table 6

Standard concentrations of different polyphenols used in this study

Standard	Concentration (mg/L)						
Catechin	10	20	30	40	50		
Epicatechin	20	40	60	80	100		
Epigallocatechin Gallate	40	84	88	120	130	200	208
Epicatechin gallate	10	20	30	40	50		
Epigallo catechin	10	20	30	40	50		

Instrumentation

Catechin, epicatechin, epigallocatechin gallate, Epicatechin gallate and Epigallo catechin were separated and quantified by HPLC according to the method reported by Kafley (2008). The instrument used was a Waters HPLC (Figure 4) equipped with 1525 binary pump, 717-plus auto sampler (Figure 5) and 2996 photodiode array detector. Empower 2 was the software used to control the instrument. Polyphenols were separated by reverse phase chromatography using a Waters radial compression, 10 cm x 8 mm C_{18} Novapak guardpak in an RCM-100 radial compression module column as the stationary phase. The mobile phase consisted of a gradient flow of 0.5% glacial acetic acid in 99.5% Milli-Q water (solvent A) and 40% acetonitrile, 0.5% glacial acetic acid in 59.5% Milli-Q water (solvent B) according to the program described in the Table 7. Flow rate was 2 ml/min for a total run time of 36 minutes.

Sample peaks were detected with a photodiode-array detector at 270 nm. The injection sample volume was set at 25 µl.

Table 7

Mobile phase gradient flow ramp

Time (min)	Flow (ml/min)	Solvent A (%)	Solvent B (%)
0-30	2	100	0
30-31	2	0	100
31-36	2	100	0

Figure 4: Waters HPLC used in this study.

Figure 5: Waters HPLC-717 plus auto sampler used in this study

Data collection and analysis procedures

Analysis of each sample was conducted in triplicate. Retention times and areas of the peaks on the chromatograms for the standards and the samples were determined using Empower 2 software. A standard curve was generated using Logger pro 3.8.2, Vernier software with areas on the Y-axis and concentration of the standards on the X-axis. The concentrations of analytes from each sample were determined by interpolation on the standard curves shown in Figures 14-18.

The mean and standard deviation of analytes were calculated using Microsoft Excel,Windows XP. Differences between the means of each analyte were evaluated for significance by one sample, t-test at $p<0.05$ (SPSS PASW Statistics 18). Individual means of all organic and non-organic teas were evaluated for significance by two sample, t-test at $p<0.05$ (SPSS PASW Statistics 18).

Measurement of fluoride content using fluoride ion selective electrode

Standard preparation

A 220mg sample of anhydrous sodium fluoride was placed into a 1000 ml volumetric flask and diluted with Milli-Q water to 1000 ml. The resulting fluoride solution was further diluted 1:10 in 900ml of Milli-Q water in a 1000 ml volumetric flask. In the final fluoride standard solution, 1.00 ml solution contained 100.0 μg F^-. From the standard solution, a series of standards were prepared by diluting 5.0, 10.0, and 20.0 ml of fluoride standard solution to 100 ml with Milli-Q water. These standards are equivalent to 0.5, 1.0, and 2.0 mg F^-/ml (Ion-selective electrode method, 2005).

Buffer preparation

In a one liter beaker, 57 ml of glacial acetic acid, 58 g NaCl, and 4.0 g 1,2 cyclohexylenediaminetetraacetic acid (CDTA) was dissolved in 500 ml Milli-Q water. The beaker was placed in a 25^oC water bath and pH was adjusted to 5.4$\pm$0.1 with 6N NaOH (approximately 125 ml). This solution was transferred to a one liter volumetric flask and diluted to 1000 ml with Milli-Q water (Ion-selective electrode method, 2005).

Equal volumes (25 ml) of sample and buffer solutions poured into a beaker. Fluoride and the reference electrodes were immersed in the buffered sample and the electrical potential was measured while stirring on a magnetic stirrer (Ion-selective electrode method, 2005).

Figure 6: Showing the fluoride ion selective electrode

Data collection and analysis procedures

Analysis of fluoride in each sample was conducted in triplicate. Fluoride standards and samples were measured to obtain millivolt reading using fluoride ion selective electrode (Figure 6). The standard curves were generated using Logger pro 3.8.2, Vernier software with millivolt readings on the Y-axis and concentration of fluoride on the X-axis. The concentration of fluoride in each sample was determined by interpolation on the standard curves shown in Figure 7, 8 & 9.

Mean and standard deviation of fluoride content were calculated using Microsoft Excel, Windows XP. Differences between the means of fluoride content were evaluated for significance by one sample, t-test at $p<0.05$ (SPSS PASW Statistics 18). Individual means of all organic and non-organic teas were evaluated for significance by two sample, t-test at $p<0.05$ (SPSS PASW Statistics 18).

Chapter IV: Results and Discussion

This chapter includes all the data and results of polyphenol extractions from organic and non-organic green tea samples by reverse phase HPLC (Kafley, 2008) and fluoride content using ion selective electrode method (Ion-selective electrode method, 2005).

Fluoride Content

Fluoride content (Table 8) was estimated by converting mV measurements to mg/L using standard calibration curves in Figures 7, 8 & 9. Results in F^-/L of tea were converted to F^-/sample size by dividing by 4 to give F^-/250 ml. These results were also converted to F^-/g tea leaves because tea bags contained different amount of leaves.

Fluoride content in the tea samples used in this study ranged from 0.10 to 0.84 mg F^-/serving tea. Non-organic green tea samples: Tropical green tea, Yogi green tea and Lipton green tea had fluoride contents of 0.22, 0.30 and 0.59 mg F^-/serving tea respectively. Organic green tea samples: Organic tulsi green tea, Revolution organic green tea, Dancing organic green tea and Organic China green tea had fluoride contents of 0.15, 0.54, 0.58 and 0.84 mg F^-/serving tea respectively. Organic white tea had the lowest fluoride content per serving (0.10 mg) and Organic china green tea had the highest fluoride content per serving (0.84 mg). Overall non-organic green teas had a fluoride content of 0.22-0.59 mg F^-/tea serving, and organic green teas had 0.15-0.84 mg F^-/tea serving.

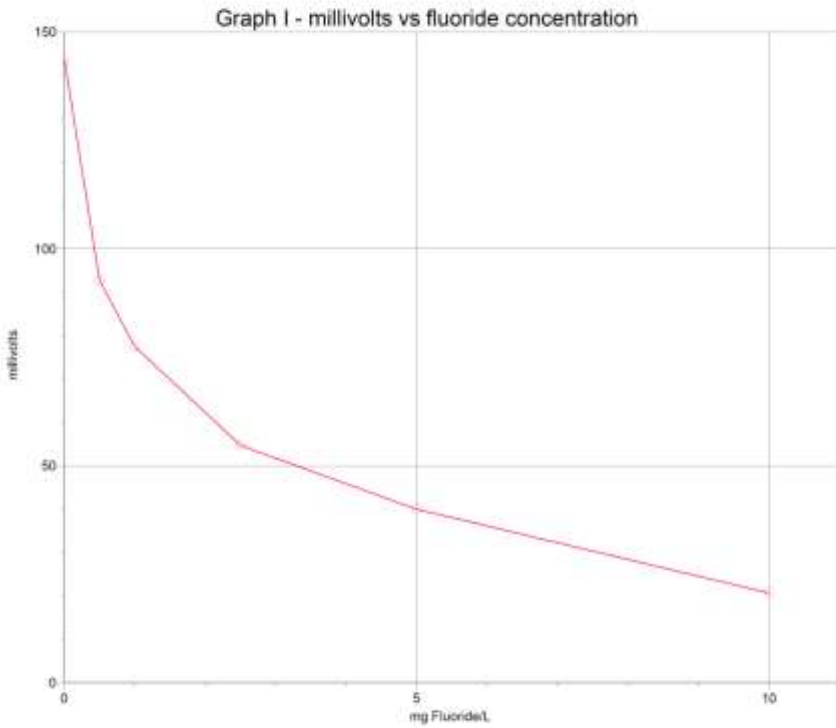

Figure 7: Calibration curve (I) of fluoride standards

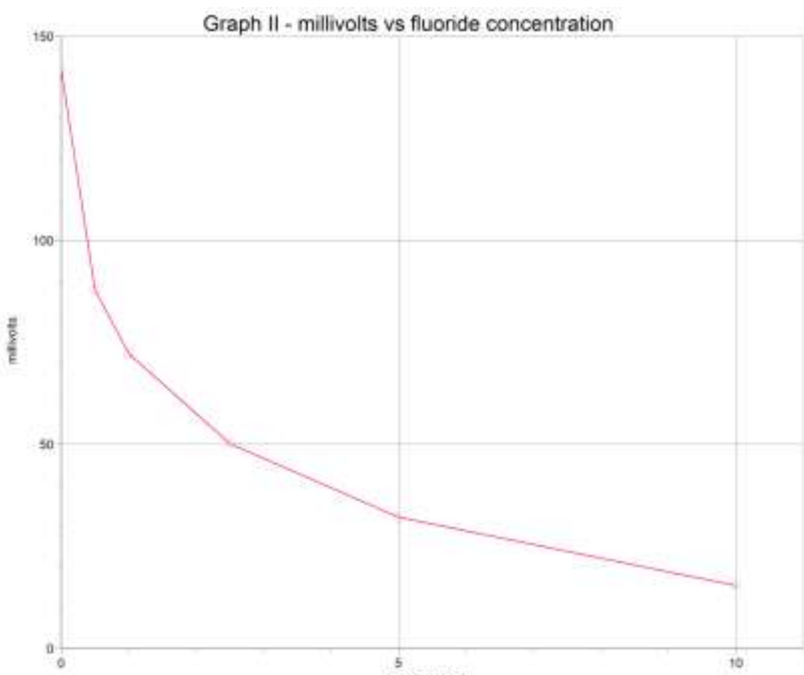

Figure 8: Calibration curve (II) of fluoride standards

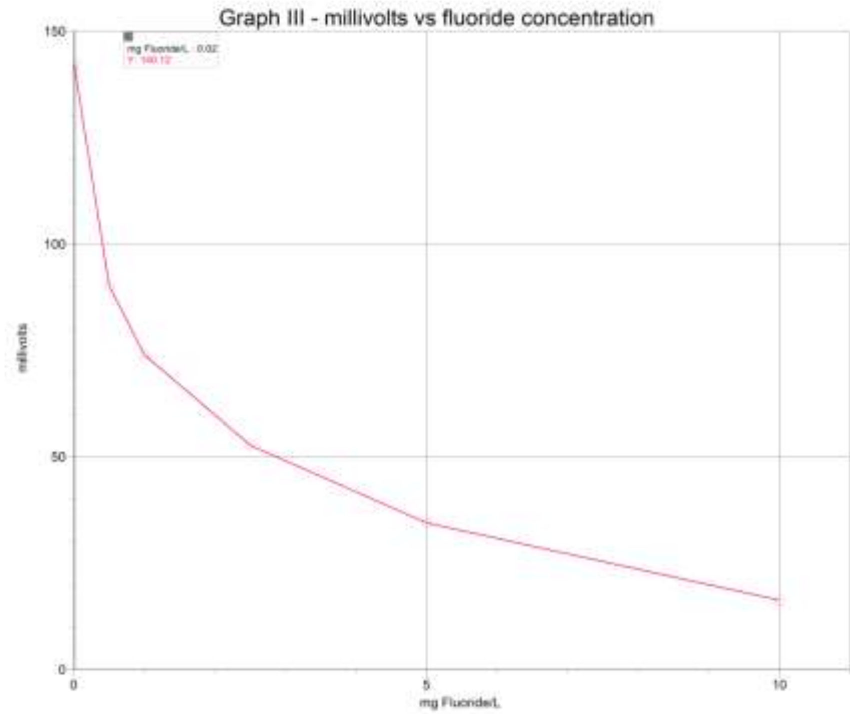

Figure 9: Calibration curve (III) of fluoride standards

Table 8

Average fluoride concentration of tea reported as mg F^-/ serving tea and mg F^-/ g tea leaves.

	Brand of Tea	mg F^-/serving tea	mg F^-/g tea leaves leaves
Organic Green Tea	Dancing	0.58^x	0.29^a
	Revolution	0.54^x	0.24^a
	China	0.84^y	0.47^b
	Tulsi	0.15^z	0.08^c
	Average	0.53^m	0.27^k
Non-organic Green Tea	Lipton	0.59^y	0.37^b
	Yogi	0.30^x	0.16^a
	Tropical	0.22^z	0.10^c
	Average	0.37^m	0.21^k
	White (organic)	0.10^z	0.05^c

‘x’ is significantly different from ‘y’ and ‘z’ at $p<0.05$.

‘a’ is significantly different from ‘b’ and ‘c’ at $p<0.05$.

Overall average fluoride content in organic green teas and non-organic green teas were 0.53 mg/serving tea and 0.21 mg/ serving tea. Averages were not significantly different at $p<0.05$.

Organic tulsi green tea (0.15 mg F^-/serving tea), Organic white tea (0.10 mg F^-/ serving tea) and Tropical green tea (0.22 mg F^-/serving tea) were significantly lower in fluoride than all other tea samples in this study. Organic china green tea (0.84 mg F^-/ serving tea) and Lipton green tea were significantly higher in fluoride (0.59 mg F^-/serving tea) than all other tea samples. Fluoride content in the non-organic tea samples: Yogi green tea was not significantly different from the organic tea samples: Dancing and Revolution green tea. In addition, all the non-organic green tea samples were significantly different from one another. Non-organic green tea samples had a narrower range of fluoride content (0.22-0.59 mg F^-/ serving tea) than the organic green tea samples (0.15-0.84 mg F^-/ serving tea).

The increasing order of fluoride content in tea samples was as follows, organic white tea (0.05 mg F^-/g tea leaves), organic tulsi green tea(0.08 mg F^-/g tea leaves), Tropical green tea(0.10 mg F^-/g tea leaves), Yogi green tea(0.16 mg F^-/g tea leaves), Revolution organic green tea (0.24 mg F^-/g tea leaves), Dancing organic green tea(0.29 mg F^-/g tea leaves), Lipton green tea(0.37 mg F^-/g tea leaves) and organic China green tea(0.47 mg F^-/g tea leaves). This wide variation of fluoride concentration in tea leaves may be due to differences in conditions like processing, age, type, selection, post harvest technique as well as cultivation between commercial tea brands. Clearly cultivation has the greatest effect on fluoride content since it is concentrated from water and the soil. As an element there is little that can be done other than steeping to remove F^-.

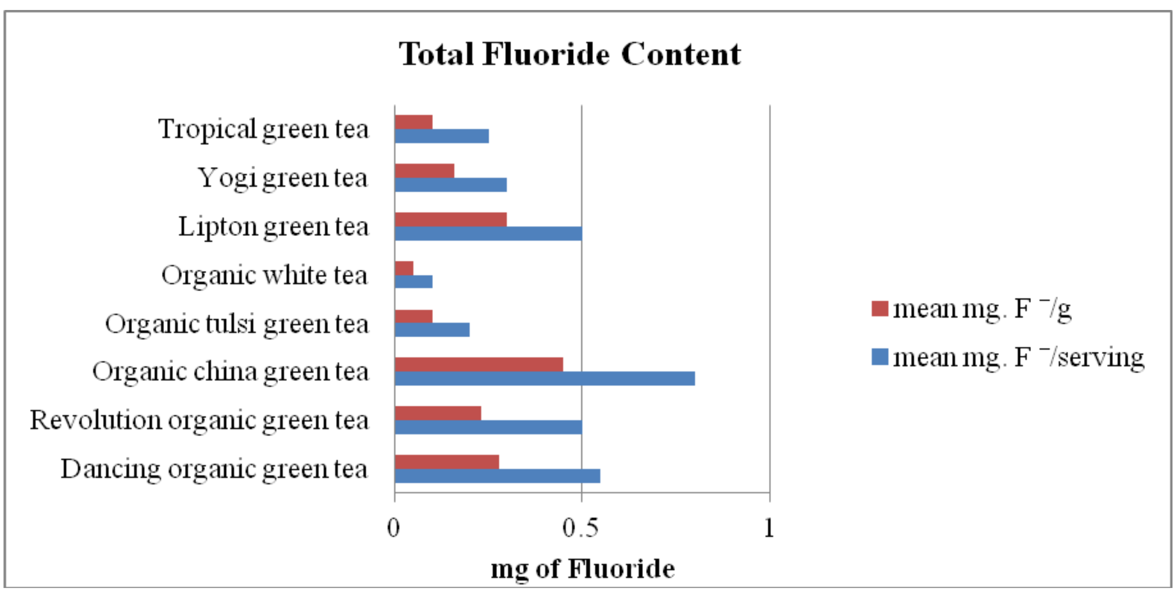

Figure 10: Fluoride content of the tea samples reported as mg F^-/g tea leaves and mg F^-/serving.

As seen in Figure 10, Organic tulsi green tea (0.08 mg F^-/g tea leaves) had the lowest fluoride content of the non-organic green tea and Organic China green tea (0.47 mg F^-/g tea leaves) had the highest fluoride content of the non-organic green tea ($p<0.05$). The fluoride results in the tea samples suggest that organic brands of green tea can vary in amount of fluoride and organic green teas do not necessarily have lower fluoride contents than non-organic green teas.

As mentioned in Kafley (2006), white tea is from buds or young leaves and green tea is from mature leaves of the tea plant. According to Wong, Fung and Carr (2003) fluoride accumulates mainly in leaves and increases with age of the leaves. This is clearly evident from the results of fluoride results in this study, where the Organic white tea had the lowest level compared to green tea samples.

Zerabruk et al. (2010) reported that fluoride in commercial green tea samples ranged from 0.11-0.19 mg F^-/(g tea leaves). Fluoride in green teas tested in this study ranged from 0.05-0.45 mg F^-/(g tea leaves). According to the review article by Wong et al. (2003), the fluoride in green tea samples ranged from 1.2-1.7 mg F^-/L tea. In the present study the fluoride ranged from 0.4-3.15 mg F^-/L tea.

Polyphenol Content

Figures 11 & 12, show the standard and tea sample chromatograms with the retention times of individual polyphenols. Individual polyphenols were identified using the retention times reported by Kafley (2008) and are shown in Table 9. Compounds were quantified by converting area measurements of individual elution curves to mg/L using standard calibration curves (Figures 13, 14, 15, 16 & 17). Table 10, lists the individual and total polyphenol contents in mg/L. Polyphenol concentrations were converted to mg/250 ml by dividing mg/L by 4, as 250 ml is the recognized serving size for tea. Because there was a difference between the weights of the tea in the tea bags from sample to sample, polyphenols were also calculated in mg/g tea leaves by dividing mg/250 ml by weight of tea, to compare between tea varieties.

Table 9

Retention times of individual polyphenols

S.No	Polyphenol	Retention time (min)
1	EGC	17.764
2	Catechin	18.140
3	EC	20.671
4	EGCG	20.906
5	ECG	23.815

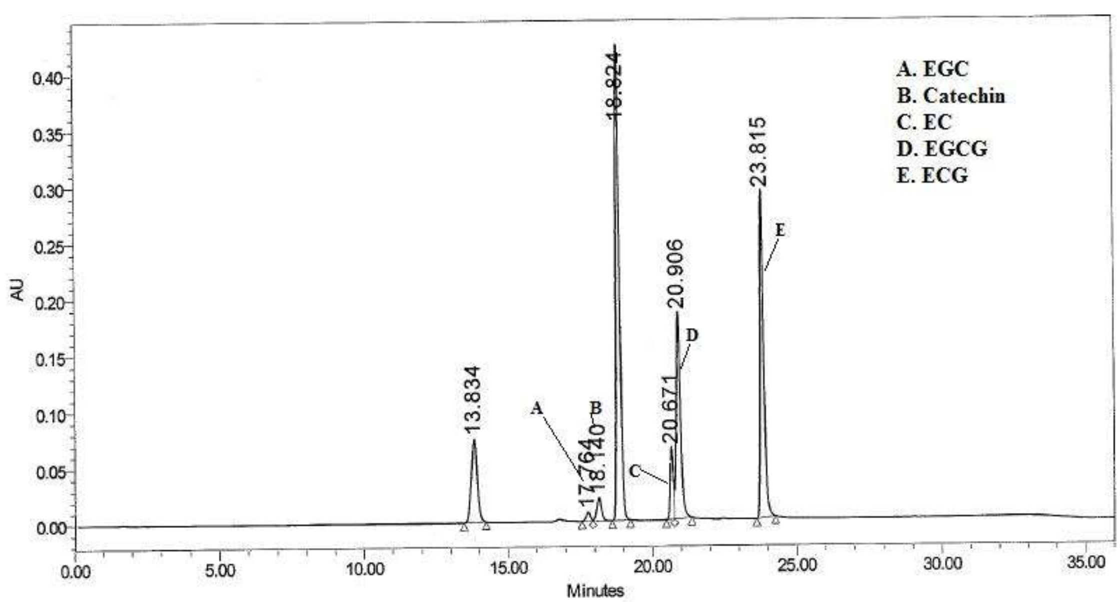

Figure 11 : *C*hromatogram of polyphenols standard solution

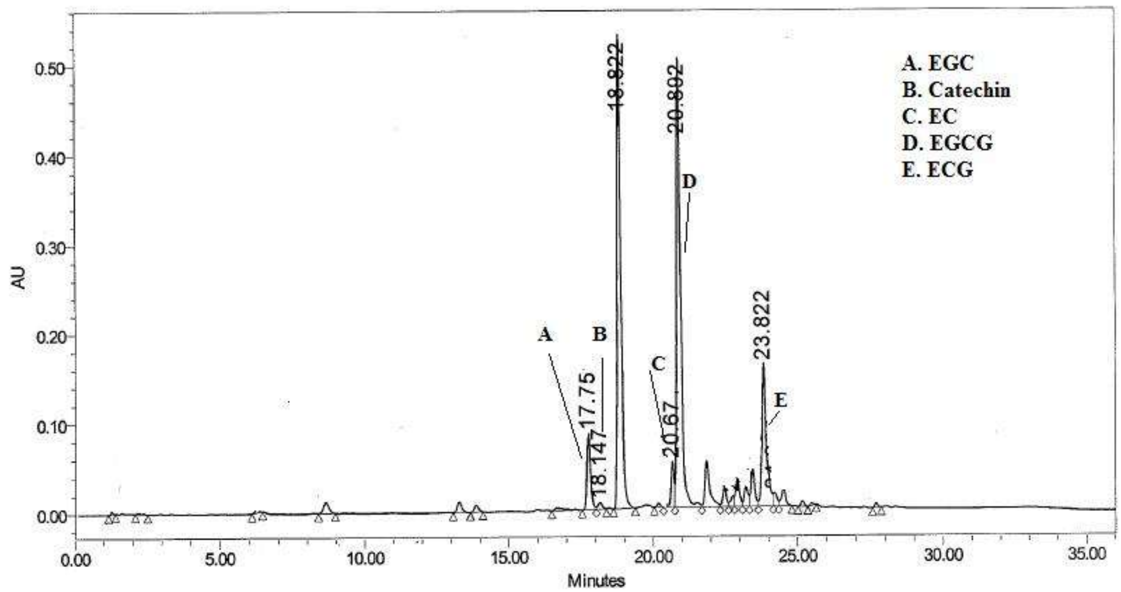

Figure 12: Sample chromatogram of the brewed tea

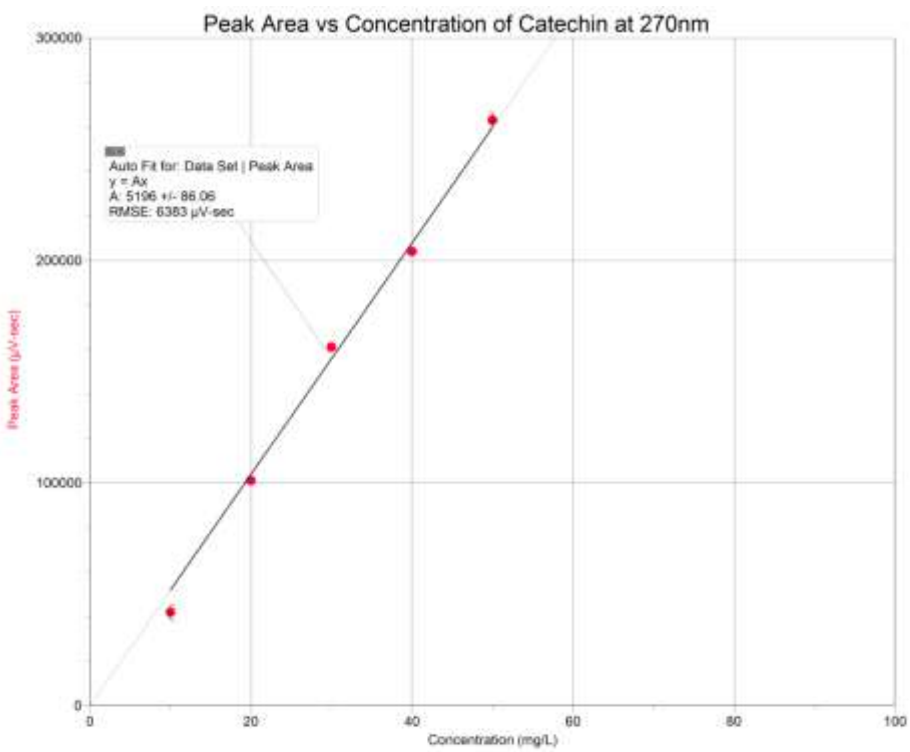

Figure 13: Standard calibration curve for catechin

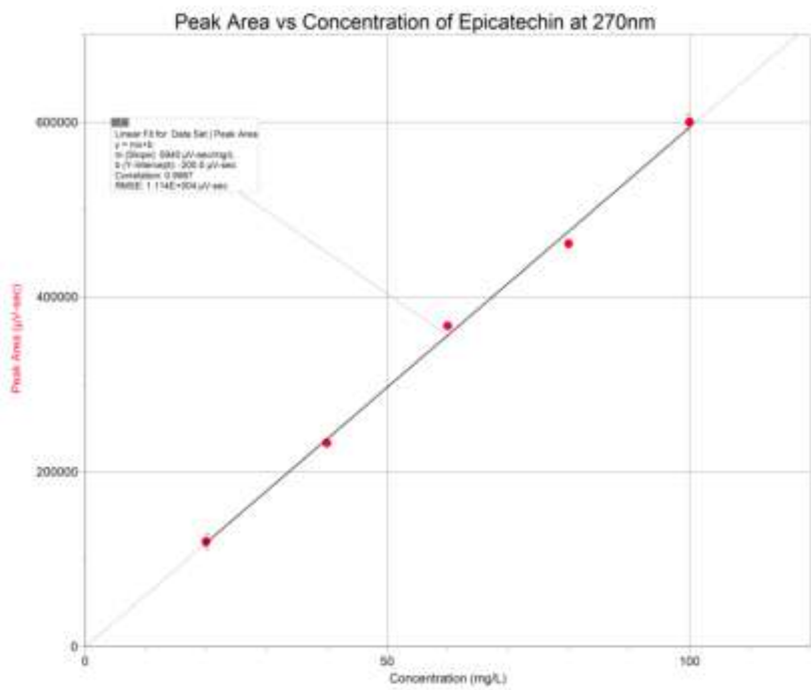

Figure 14: Standard calibration curve for Epicatechin

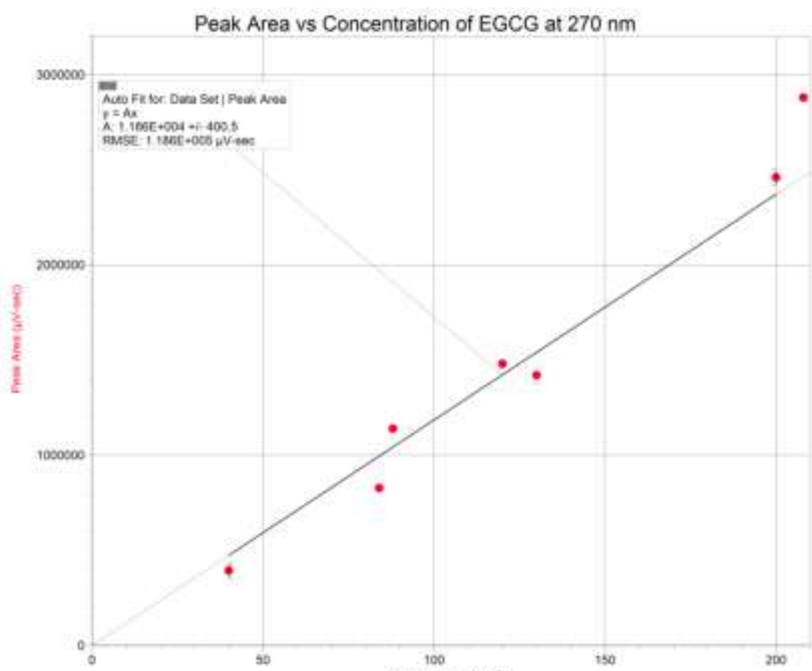

Figure 15: Standard calibration curve for EGCG

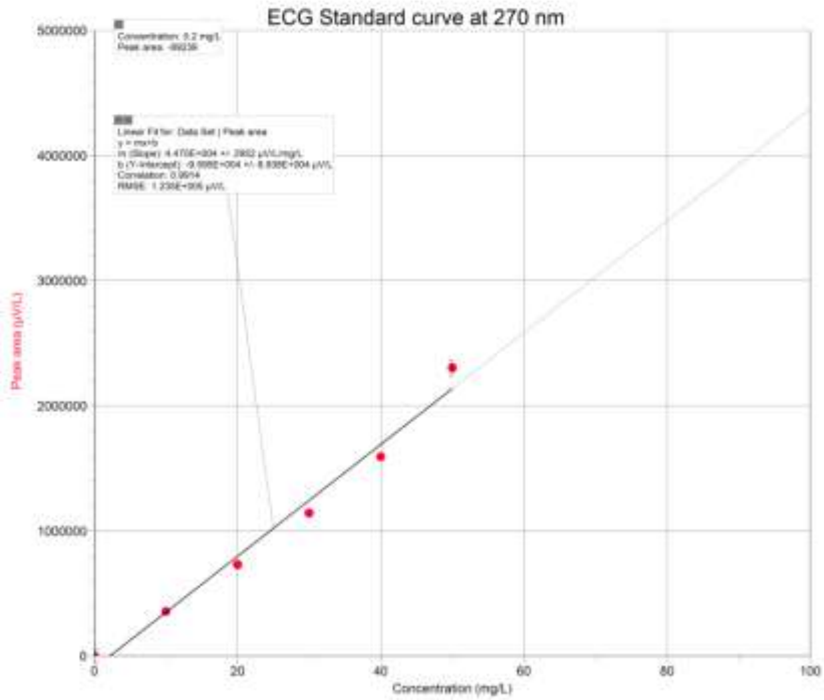

Figure 16: Standard calibration curve for ECG

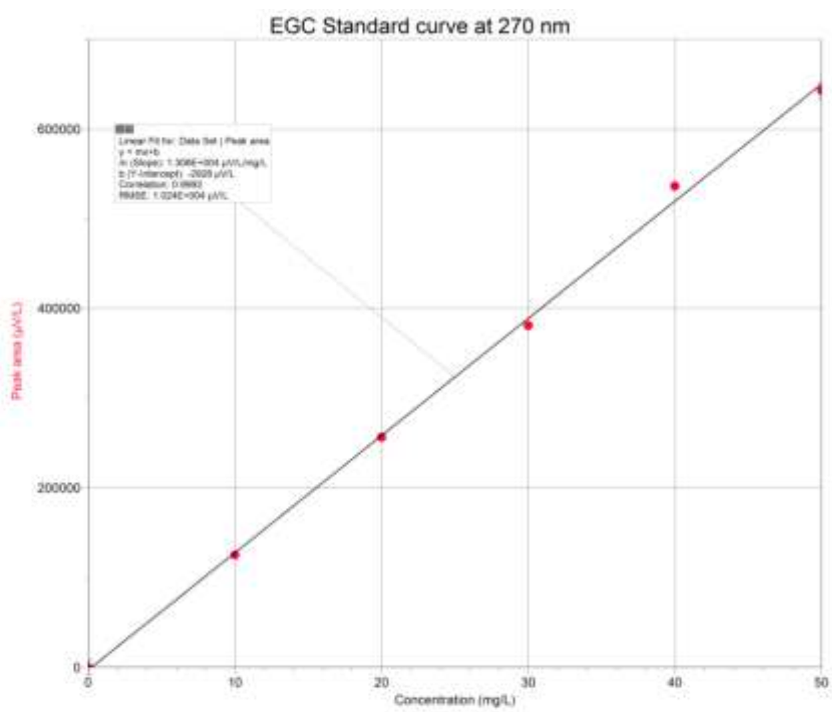

Figure 17: Standard calibration curve for EGC

Total polyphenol content in the tea samples used in this study ranged from 66.7 to 185.7 mg/serving tea (Table 10). Green tea samples: Yogi green tea, Lipton green tea and Tropical green tea had total polyphenol content of 66.7, 142.1 and 159.5 mg/serving tea respectively. Organic green tea samples: Organic tulsi green tea, Dancing organic green tea, Organic china green tea and Revolution organic green tea, had 98.1, 149.9, 163.9, 164.5 mg/serving tea respectively. Organic white tea had the highest total polyphenol content per serving, 185.7 mg and Yogi green tea had the lowest total polyphenol content per serving, 66.7 mg.

Table 10

Distribution of individual polyphenols in organic and non-organic green teas in mg/L.

	Brand of Tea	C	EC	EGCG	EGC	ECG	TP
Organic Green Tea	Dancing	14.2±0.4	68.7±2.4	416.3±6.4	59.3±3.2	41.0±2.8	599.5±15.2
	Revolution	11.7±0.4	61.7±1.2	478.3±7.6	60.3±0.8	46.2±2.4	658.2±12.4
	China	19.7±1.2	61.5±0.8	467.0±4.0	56.8±2.4	50.5±3.2	655.5±11.6
	Tulsi	17.2±0.8	27.8±0.8	281.3±2.4	20.3±0.4	45.2±3.2	391.8±7.6
Non-organic Green Tea	Lipton	17.0±0.4	45.3±1.6	415.8±3.6	48.0±3.2	42.6±3.6	568.7±12.4
	Yogi	2.7±0.4	23.0±1.6	195.3±3.2	21.8±1.2	23.6±2.0	266.4±8.4
	Tropical	10.7±0.4	57.3±2.8	468.5±4.8	41.8±1.2	59.5±1.2	637.8±10.4
	White(organic)	11.5±0.4	39.0±0.4	598.2±10.4	25.8±3.2	68.2±1.2	742.7±15.6

C=Catechin, EC=Epicatechin, EGCG=Epigallocatechin gallate, EGC=Epigallo catechin, ECG=Epicatechin gallate,

TP=Total polyphenol (C+EC+EGCG+EGC+ECG)

Comparison of total polyphenol content

The average total polyphenol content in organic green teas was 73.8 mg/g tea leaves. The average total polyphenol content in non-organic green teas was 65.5 mg/g tea leaves. However, there was no significant difference beween the polyphenol content in organic and non-organic teas groups.

Total polyphenols in Organic tulsi green tea (98.1 mg/ serving tea) and Yogi green tea (66.7 mg/ serving tea) were significantly lower than the other tea samples used in this study. Organic white tea (185.7 mg/ serving tea) had a significantly ($p<0.05$) higher total polyphenol content than other tea samples. Total polyphenol content in the non-organic tea samples; Lipton green tea and Tropical green tea, were not significantly different from the organic tea samples; Dancing organic green tea, Revolution organic green tea and Organic china green tea.

When evaluated as mg/(g tea leaves), the total polyphenol content of Organic white tea was not significantly higher than other tea samples used in this study, but Lipton green tea was. From Table 11, total polyphenols in Organic china green tea and Lipton green tea were significantly higher, and Yogi green tea was significantly lower ($p<0.05$) when compared with other tea samples. The increasing order of total polyphenol in tea samples was as follows: Yogi green tea (35.1 mg/g tea leaves), Organic tulsi tea (54.6 mg/g tea leaves), Tropical green tea (72.5 mg/g tea leaves), Revolution organic green tea(74.8 mg/g tea leaves), Dancing organic green tea (74.9 mg/g tea leaves), Organic white tea (86.3 mg/g tea leaves), Lipton green tea (88.8 mg/g tea leaves), Organic china green tea (91.0 mg/g tea leaves). This wide variation of polyphenol concentration in tea leaves may be due to differences in conditions like processing, age, type, selection, post harvest technique as well as cultivation between commercial tea brand

Table 11

Total polyphenol content in organic and non-organic green teas in mg/serving tea and mg/g tea leaves.

	Brand of Tea	TP/serving tea leaves	TP/g tea leaves
Organic Green Tea	Dancing	149.9^{x}	74.9^{a}
	Revolution	164.5^{x}	74.8^{a}
	China	163.9^{x}	91.0^{b}
	Tulsi	98.1^{z}	54.6^{c}
	Average	-	73.8^{m}
Non-organic Green Tea	Lipton	142.1^{x}	88.8^{b}
	Yogi	66.7^{z}	35.1^{c}
	Tropical	159.5^{x}	72.5^{a}
	Average	-	65.5$^{m'}$
	White (organic)	185.7^{y}	86.3^{a}

'x' is significantly different from 'y' and 'z' at $p<0.05$.

'a' is significantly different from 'b' and 'c' at $p<0.05$.

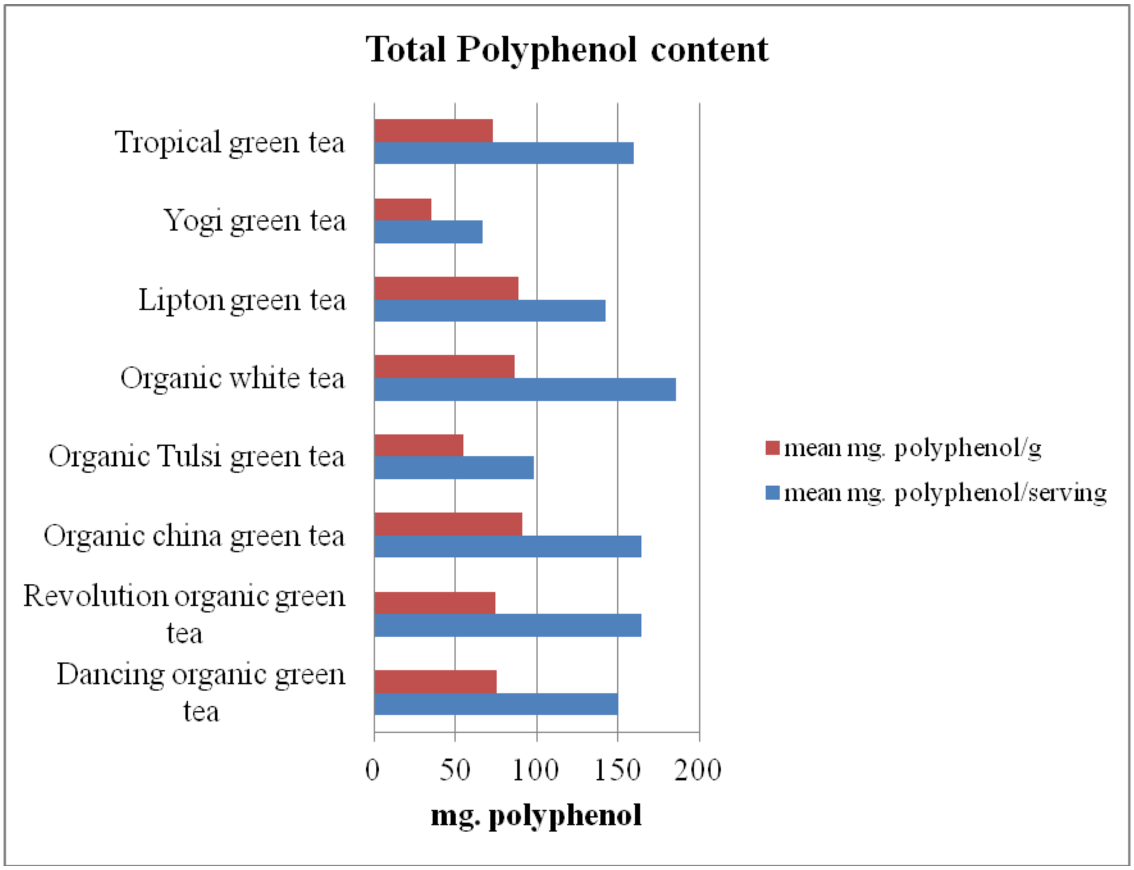

Figure 18: Total polyphenol content of the tea samples in mg/g tea leaves and mg/serving tea.

As seen in Figure 18, Yogi green tea has lowest total polyphenol content (35.1 mg/g tea leaves) of any other tea used in this study. Lipton green tea had significantly higher total polyphenol content (88.8 mg/g tea leaves) compared to Dancing organic green tea (74.9 mg/g tea leaves), Revolution organic green tea (74.8 mg/g tea leaves), Organic tulsi green tea (54.6 mg/g tea leaves). Thus the total polyphenol results in the tea samples suggest that organic varieties of green teas can have wider range of total polyphenol contents.

In a study conducted by Kafley (2008), on caffeinated and decaffeinated green tea, the total polyphenol content ranged from 52.4-239 mg/serving tea and 88.0-143.0 mg/serving tea respectively. In the current study the total polyphenol content in organic green tea and non-organic green tea ranged from 98.1-164.5 mg/serving tea and 66.7-159.5 mg/serving tea respectively. Friedman et al. (2005) conducted similar research using 77 teas available in the United States. The total polyphenol content in organic green tea, flavored organic green tea and non-organic green tea ranged from 14.2-100 mg/(g tea leaves), 19.9-36.0 mg/(g tea leaves) and 4.4-87.1 mg/(g tea leaves) of tea respectively. In the current study the total polyphenol content in organic green tea and non-organic green tea ranged from 54.6-91.0 mg/(g tea leaves) and 35.1-88.1 mg/(g tea leaves) of tea respectively. Total polyphenol content in a White tea estimated by Friedman et al. (2005) was 13.4 mg/(g tea leaves). In the current study Organic white tea contained 86.3 mg/(g tea leaves). The reasons for this difference could be due to age of tea, soil, season of harvesting, which will have effect on the total polyphenol content.

Chapter V: Conclusion

The objectives of this study were to compare fluoride and polyphenol contents in organic and non-organic varieties of green tea and to determine if the organic or non-organic designation of green tea is a reliable predictor of polyphenol or fluoride content in commercially available green tea. Fluoride content was measured using Fluoride Ion-selective electrode and polyphenols by reversed phase HPLC.

Fluoride content in tea samples varied widely from sample to sample. Organic white tea had the lowest fluoride content followed in increased order by Organic tulsi green tea, Tropical green tea, Yogi green tea, Revolution organic green tea, Dancing organic green tea, Lipton green tea and Organic China green tea, which had the highest fluoride content. Fluoride content in organic and non-organic green tea varieties suggests that the fluoride in tea has no relationship to organic or non-organic farming practices. It may be depend upon several other factors like polluted air, water, soil and fertilizers used in the farming. White tea is a better choice because it uses only very young leaves from the tea plant and contains the least amount of fluoride among all the tea types.

Non-organic green teas had a wider range in total polyphenol content, 35.1-88.8 mg/(g tea leaves), compared to organic green tea, 54.6-91 mg/(g tea leaves). Yogi green tea had the lowest total polyphenol content followed in increasing order by Organic Tulsi green tea, Tropical green tea, Revolution organic green tea, Dancing organic green tea, Organic white tea, Lipton green tea and Organic china green tea, which had the highest amount. Polyphenol content in Organic China and Lipton green teas were significantly higher ($p<0.05$) than any other tea used in this study. Polyphenols in Organic Tulsi and Yogi green teas were significantly lower ($p<0.05$) than any other tea used in this study. The total polyphenol content may depend on nature and origin of the seeds, soil, water and fertilizers used during the farming. While one cannot use the results of this study to determine the effects of organic and non-organic farming practices on polyphenol levels in tea leaves, it does show that purchasing organic tea does not guarantee higher polyphenol levels. There is evidence in the literature that organic practices result in higher levels of bioactive chemicals like antioxidants, but the results of this study suggest that other factors in the commercial supply chain for example blends of cultivars, age, process conditions, and packaging might have a greater impact on polyphenol content than organic farming practices. Purchasing organic green tea does not guarantee higher levels of polyphenols.

Recommendations

1) In this study, I have used a single temperature and time for the extraction of tea components. I would recommend extraction of fluoride and polyphenols at different temperatures and times to identify optimum conditions for the extraction of specific components.

2) To find any sensory differences, sensory analysis of organic and their non-organic green tea counter parts will be an interesting study.

3) In this study organic and non-organic green teas from different brands and origins were used to determine relative amounts of polyphenols and fluoride in commercial tea products. Comparing like varieties of organic and non-organic tea from single regions and growing cycles would be more informative in determining causal relationships between bioactive ingredients and inorganic elements and agricultural practices.

4) In this study I have selected organic and non-organic green teas of different commercial brands. Conducting the experiments with same commercial brand of organic and organic green tea might yield definite conclusions about organic and non-organic farming on fluoride and polyphenol concentrations.

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