Effect of Extraction Parameters on Polyphenols of Caffeinated and Decaffeinated Green Tea

by

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ABSTRACT

Green tea is of growing importance due to its health benefits associated with it's antioxidant contents. The extraction parameters, time and temperature, can influence the polyphenol and methylxanthine concentrations of green tea, and were investigated in this study. The purpose of the study was to quantify the polyphenols (catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate) and methylxanthines (caffeine, theobromine and theophylline) in different green teas (Salada®, Celestial Seasonings®, and Bigelow®) using both caffeinated and decaffeinated forms manufactured commercially in United States at different extraction times (2, 4, 6, 8, and 10 minutes) and extraction temperatures (80°C , 85°C , 90°C, 95°C, 100°C). This study also quantified the concentrations at room temperature (24°C) and longer extraction times (1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour, 24 hour).

For the longer time extraction conditions, the amount of polyphenols increased up to 7 hours and then decreased in concentration after 24 hours for all the brands both in caffeinated and decaffeinated forms. Larger concentrations of polyphenols and methylxanthines are obtained at a temperature of 100°C and at extraction time of 8-10 minutes (for example, 280 mg/serving for Celestial Seasonings® green tea at 100°C, at 10 minutes). The concentrations of polyphenols decreased with a decrease of temperature at constant times and increased with an increase of time at constant temperatures. Interestingly Bigelow® decaffeinated green tea had larger amounts of EGCG and total polyphenol content compared to the caffeinated tea, which was not found in either Salada® or Celestial Seasonings® green teas.

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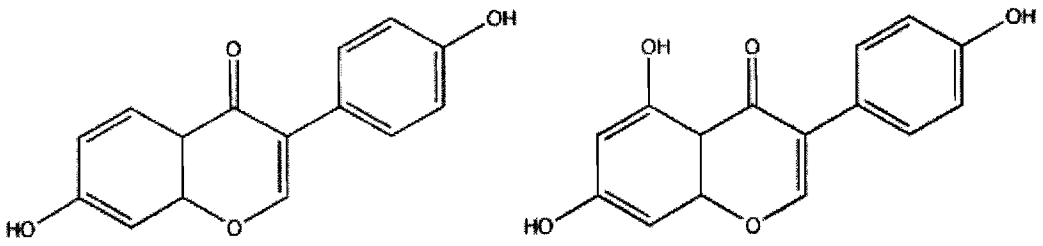
Chapter I: Introduction

Tea is mainly divided into three varieties, green, black, and oolong with the variation in the teas a result of their processing. Black tea is made from leaves that are completely fermented or oxidized after they have been dried (Pelillo et al., 2002). Oolong tea is partially fermented and falls between the black and green tea. Green tea is made from unfermented leaves. The dried unfermented green tea has green tea catechins, which are more preserved than in partially fermented oolong tea or fully fermented black tea (Pelillo et al., 2002). Oxidation of the catechins in green tea is prevented by inactivation of phenol oxidases while the formation of dimeric theaflavins and polymeric thearubigins, occurs that impart the black color to black tea. These theaflavins and thearubigins are due to the phenolase catalyzed oxidation of catechins in green tea (Friedman et al., 2005). Of the most popular beverages in the world, green tea is the one known for its natural antioxidant properties (Chiu, 2006) due to its catechins.

Polyphenols are a group of chemical and natural substances present in beverages (such as tea, red wine, and grape juice) obtained from plants, fruits, and vegetables (Reznichenko, Amit, Youdim & Madel, 2005). Flavonoids are polyphenolic compounds that include the subclasses of flavanones, flavones, isoflavones, flavanols (flavans), flavonols and anthocyanins (Li & Jiang, 2007). The chemical structure of isoflavones (3-phenyl-4*H*-1-benzopyr-4-one) consists of two benzene rings with the rings linked to by a heterocyclic pyrane ring (Chen & Anderson, 2002). One hydroxyl group (-OH) is attached to each benzene ring. The two most important isoflavones are genistein and daidzein (Figure 1).



Daidzein Genistein

Figure 1. Diadzein and genistein structures

Isoflavone differs from flavones in the position of the phenyl group on the 4*H*-1-benzopyr-4-one, in isoflavone this occurs at position 3 relative to the oxygen of the ring, and in flavones the phenyl group is at position 2 (Figure 2).

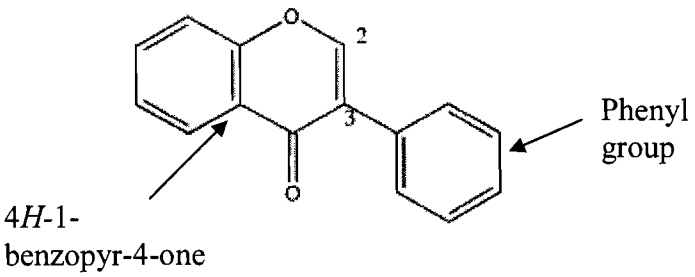


Figure 2. Chemical structure of the isoflavone backbone (3-phenyl-4*H*-1-benzopyr-4 one)

(Chen & Anderson, 2002)

Generally, flavonoids were considered derivatives of 2-phenylchromone, the parent compound, which is composed of three phenolic rings and are referred as A-, B-, C-rings (Li & Jiang, 2007). All of the rings contain varying levels of hydroxylation and methoxylation.

Flavans (Flavanols) are composed of the catechins that are analyzed in this study (Li & Jiang, 2007). Many of the flavonoid compounds have been shown to have a number of physiological benefits, mainly in cognitive functions and prevention of memory impairment.

Methylxanthines, which include caffeine, theobromine and theophylline, were also analyzed in this study. Over consumption of caffeine could result in anxiety and insomnia mainly in sensitive individuals (Lee, Park, Kim & Kim, 2007). Caffeine also has beneficial properties such as the use by athletes to increase performance before competition. To obtain caffeine-free products, the decaffeination process has been commercialized. Decaffeination processes like extraction with dimethyl chloride, ethyl acetate and supercritical CO_2 have been introduced in commercial processing. It has been discovered that the decaffeination process may reduce the amount of polyphenols in tea, so it might not provide the same health benefits as caffeinated green tea.

Green tea is one of the most widely consumed teas (Lee et al., 2007). Green tea is highly preferred not only due to its purported health benefits but also due to its aroma and taste. Brewed green tea contains slightly fruity and greenish aroma notes. It has been proven that most volatile compounds decrease by the decaffeination process using supercritical CO_2 process (Lee et al., 2007). Due to the antioxidant properties of its polyphenol content many studies concerning green tea effects have been targeted at its possible cardiovascular, anti-inflammatory, and anticarcinogenic effects (Silvia, Orly, Tamar & Moussa, 2004). (-)- Epigallocatechin gallate (EGCG) is the most abundant and investigated catechin of green tea polyphenols. Among the catechins of green tea, EGCG is shown to exhibit important anti-cancer properties and also plays a role in cancer prevention ("New Cancer Research," n.d.). Green tea extract is known for its cancer-preventive behavior in humans in Japan (Sachiko et al., 1997). Drinking green tea is shown to be protective against colon cancer in humans (Suminori, 1992).

The main polyphenols of green tea are flavan-3-ols (catechins) and their corresponding gallate compounds, which constitute about one-third of the dry weight of tea leaves (Kia, Chi, Micheal, Kwong & Chi, 2004). Polyphenols of green tea analyzed in this investigation include

catechin (C), epicatechin (EC), epigallocatechin-3 gallate (EGCG), epigallocatechin (EGC), epicatechins-3-gallate (ECG) and the methylxanthines, caffeine (Caf), theobromine (Tb) and theophylline (Tp). Depending on the type of raw material, type of varieties, climate and cultivation, the amount of green tea catechins varies (Bonoli, Pelillo, Gallina Toschi & Lecker, 2003). In addition, green tea catechins availability and concentration can also depend on the technologies applied during extraction, and the preservation process. The green tea catechins vary depending on the time and temperatures applied during extractions.

The concentrations of catechins and methylxanthines present in the caffeinated and decaffeinated green teas were quantified by using High Performance Liquid Chromatography (HPLC). Three commercial brands of green teas, namely Bigelow®, Celestial Seasoning® and Salada®, were highlighted and tested in this study. The effect of extraction parameters including time and temperature were observed on the three commercial brands of green tea in both caffeinated and decaffeinated types.

Statement of the Problem

Polyphenols and methylxanthines were investigated in three brands of green tea. Limited data on the quantifications of polyphenols and methylxanthines are available in the commercial brands chosen and also quantification of polyphenols at different time and temperature conditions chosen in both caffeinated and decaffeinated green teas is scarce or minimal.

Commercial brands analyzed included Bigelow®, Celestial Seasoning® and Salada® caffeinated and decaffeinated green teas. These green teas were tested using reverse-phase HPLC to quantify the levels of catechin (C), epicatechin (EC), epigallocatechin-3 gallate (EGCG), epigallocatechin (EGC), epicatechins-3-gallate (ECG) and the methylxanthines caffeine (Caf), theobromine (Tb), and theophylline (Tp).

The study using HPLC was conducted at University of Wisconsin-Stout Chemistry Department, third floor Jarvis Hall Science Wing from fall 2006 to spring 2007.

Purpose of the Study

The purpose of the study was to quantify flavan-3-ols (C and EC) and their corresponding gallate compounds (EGC, ECG and EGCG) and methylxanthines (caffeine, theobromine, and theophylline) by reverse-phase HPLC. The objectives were to:

1) Quantify the concentrations of C, EC, EGC, ECG, EGCG, caffeine, theobromine and theophylline in Bigelow®, Celestial Seasonings® and Salada® caffeinated and decaffeinated green teas extracted for different lengths of time (2 minutes, 4 minutes, 6 minutes, 8 minutes, 10 minutes) and at a variety of temperatures (80 °C , 85°C , 90 °C, 95 °C, 100 °C).
2) Quantify the concentrations of C, EC, EGC, ECG, EGCG, caffeine, theobromine and theophylline in Bigelow®, Celestial Seasonings® and Salada® caffeinated and decaffeinated green teas extracted at room temperature (24 °C) and longer extraction times (1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour, 24 hour).
3) Compare the polyphenol concentrations of the Bigelow®, Celestial Seasonings® and Salada® green teas as a function of time and temperature extraction conditions.
4) Compare the polyphenol concentrations of caffeinated green teas with the decaffeinated green teas at room temperature and different time points.

Assumptions of the Study

Flavan-3-ols (C and EC) and their corresponding gallate compounds (EGC, ECG and EGCG) and methylxanthines are assumed to be present in all the green teas under high-temperature and room-temperature extraction conditions. In addition, it was thought that the polyphenol concentrations would increase as the extraction time increased at any temperature. Photochemical degradation of polyphenols was expected to be negligible.

Definition of Terms

The following terms are important in understanding this study and will be used commonly throughout this research paper and are defined as follows.

Antioxidant. Antioxidants are substances that offer protection against lipid oxidation, react with free radicals, reduce oxidative stress, and stop low density lipoproteins (LDLs) or bad cholesterol from being oxidized (Lee et al., 2007).

Decaffeinated green tea. This is referring to the green tea without caffeine in it. Caffeine is removed using decaffeination method. Two decaffeinating methods exist, one is using ethyl acetate solvent and other is using water and CO_2 ("Celestial Seasonings Caffeine Free Tea," n.d).

Green tea. Tea made from leaves of *Camellis sinensis* that are steamed and dried without fermenting.

Methylxanthines. A chemical group of drugs derived from xanthine (Figure 3) (a purine derivative); members of the group include theophylline, caffeine, and theobromine and they differ from each other by the number of methyl ($-CH_3$) groups ("Methylxanthine," n.d.).

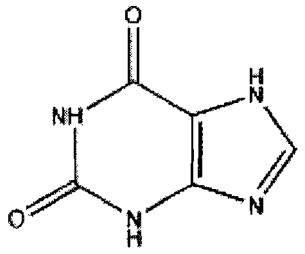


Figure 3. Xanthine (3, 7-dihydro-purine-2,6-dione)

("Methylxanthine," n.d.)

Polyphenols. According to Lazarus (2001), a compound comprised of two or more aromatic rings with each ring containing one or more hydroxyl groups (as cited in Kasper, 2006).

Chapter II: Literature Review

Camellia sinensis is one of the health promoting tea plants belonging to the family of Theaceae (Weisburger, 1996). Leaves of the tea plant are rolled, chopped and dried. Dried leaves when added to boiling water yield an extract or tea beverage. Tea was originally discovered when tea leaves fell into boiling water at a reception for Chinese Emperor Shen Nung about 2735 B.C. Green tea, oolong tea and black tea are derived from the same plant called as *Camellia sinensis* ("Tea Processing," n.d.). The difference between the teas is due to the difference in processing methods. The processing steps that are commonly used are withering, rolling, oxidation and drying. Green tea does not undergo oxidation and is steamed to oxidation making it distinctive in color and taste from black and oolong tea ("Andao," n.d.). Green tea resembles the original, untreated tea leaf in aromas and flavors. Oolong tea is also known as partially oxidized tea. The range of oxidation lies between 7.5% (slight oxidation) to 70% (complete oxidation). Black tea is fully oxidized. The dark color of black tea is due to the phenolase catalyzed oxidation of catechins in green tea (Friedman, et al., 2005). An enzyme, polyphenol oxidase, is activated during the rolling and chopping process of the tea leaves. People in China have heated the leaves of the *Camellia sinensis* plant to inactivate the polyphenol oxidase which has resulted in green tea. Polyphenols oxidized after withering for about 6 hours after chopping and rolling results in black tea. Oxidation of polyphenol oxidase for about 1 or 2 hours results in oolong tea.

In general tea, *Camellia* Sect. *Thea* is known to contain 32 species and four varieties (Yang, Ye, Xu & Jiang, 2007) and of all these *Camellia sinensis* and *Camellia assamica* are known to have good health benefits due to their antioxidative properties and the presence of bioactive substances. Both of these plants contain eight catechins with EGCG as the major and

most potent constituting about 3-13% in dry leaves. *Camellia ptilophylla* contain theobromine as a major alkaloid and contains no caffeine or a small amount of it with a small amount of theophylline (Yang et al., 2007). The amount of catechins in the leaves of *C. ptilophylla* and *C.assamica* are unknown. The amount of caffeine is shown as 2.72%, 0.94% in *Camellia sinensis* and *Camellia assamica,* respectively.

Tea is produced by processing shoots that include tender apical bud and subtending three leaves (Sharma, Gulati & Ravindranath, 2006). Infusion of leaves with hot/cold water results in a tea beverage. Processing procedures of the Chinese include roasting the tea shoots in a metal roaster and then using the unidirectional rotary roller for processing. The unidirectional roaster twists the leaves and compacts the particles. The Japanese use steaming procedures to inactivate the shoots and then process by bi-directional rolling. Bi-directional rolling makes the shoot surface flat without any twists and by spreading the leaf juice over the entire surface.

Catechins

Green tea is receiving increased interest from food scientists due to its purported antioxidant properties and health benefits. Epigallocatechin gallate (EGCG) in green tea accounts for 50-60% of the catechins; however, in black tea due to the oxidation of polyphenol oxidase EGCG drops and is about 12% of the polyphenols (Ito et al., 2003). Studies indicate that the composition of catechins changes during heat processing and storage. Under high temperature and high pH conditions, catechins undergo oxidation resulting in a rapid decrease in concentration (Ito et al., 2003). Therefore, brewing green teas at high temperatures for long period does decrease the concentrations due to the oxidation. Further research would be required to determine the time and temperatures that a decrease in concentrations would occur.

Extraction of catechins was conducted using organic solvents such as methanol and acetonitrile (Yoshida, Kiso & Goto, 1999), while other studies show an extraction utilizing hot water instead of these organic solvents. Extraction of catechins was also shown using acetonitrile-water (1:1, v/v), which was considered as an efficient extraction solvent combination (Yoshida et al., 1999). This method of extraction using organic solvents however may not reflect the actual levels of the tea catechins if studied for human consumption of tea concentrations. Therefore, extraction using water which reflects the polyphenols obtained in tea would be more efficient and practical to use.

Tea catechins exist as two geometrical isomers known as *trans* catechins and *cis* epicatechins (Friedman et al., 2005). This existence depends upon the stereochemical configuration of the 3', 4' – dihydroxyphenyl and hydroxyl groups at the 2- and 3- positions of the C-ring. Each of the *trans* and *cis* isomers in turn exists as two optical isomers namely (+)-catechin (Figure 4) and (-)-catechin (Figure 5) and and (+)-epicatechin (Figure 6) and (-)-epicatechin (Figure 7), respectively. By esterification with gallic acid, (-)-catechin can be converted to form (-)-catechin-3-gallate, epicatechin-3-gallate (Figure 9), (-)-epigallocatechin-3-gallate (Figure 10), and (-)-gallocatechin-3-gallate, respectively.

Figure 11 shows the structures of methylxanthines.

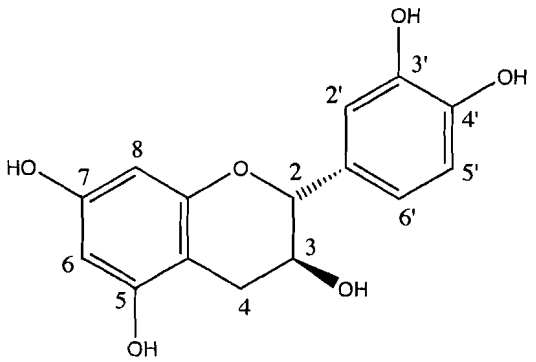


Figure 4. (+)-Catechin

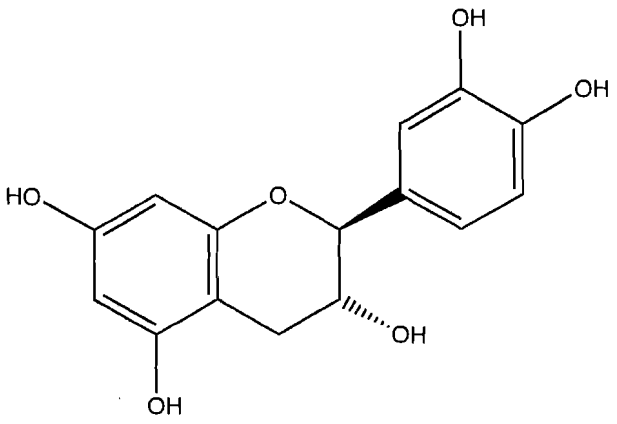


Figure 5. (-)- Catechin (C)

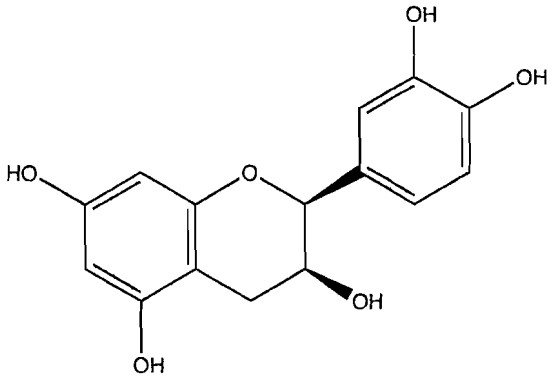


Figure 6. (+)-Epicatechin (EC)

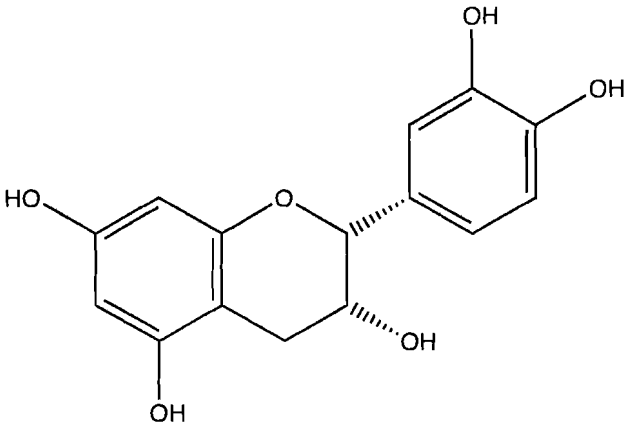


Figure 7. (-)-Epicatechin (EC)

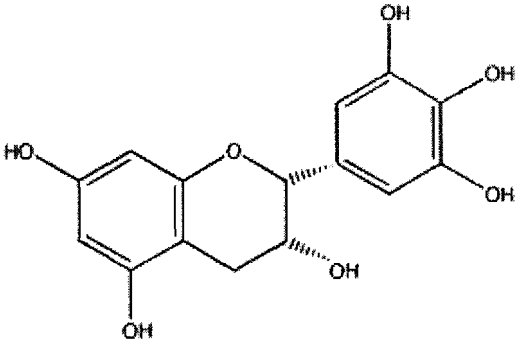


Figure 8. (-)- Epigallocatechin (EGC)

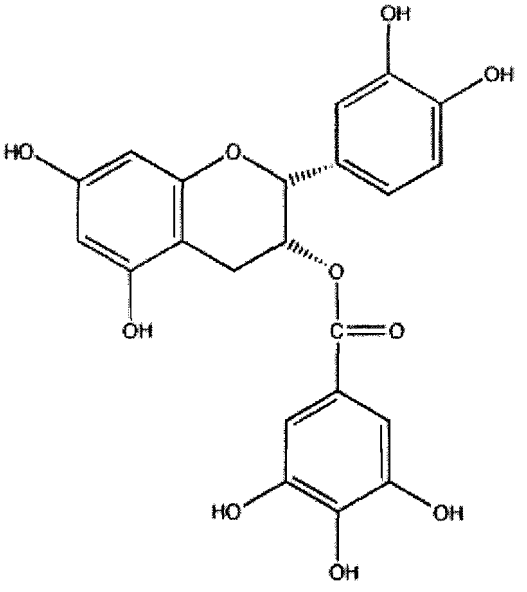


Figure 9. (-)-Epicatechin gallate (ECG)

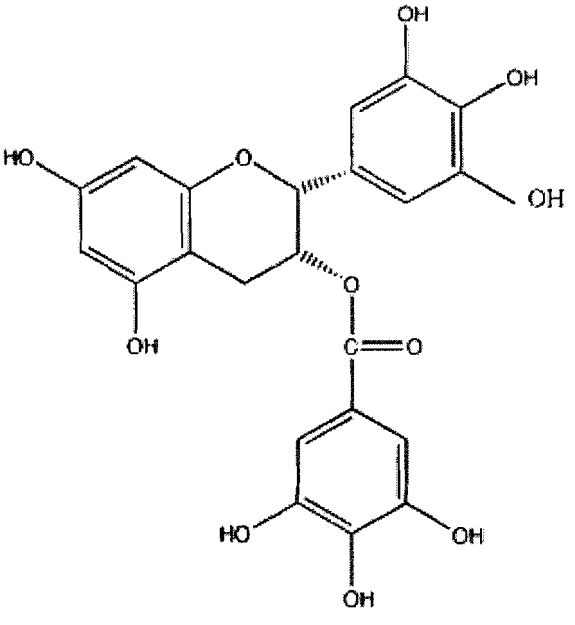
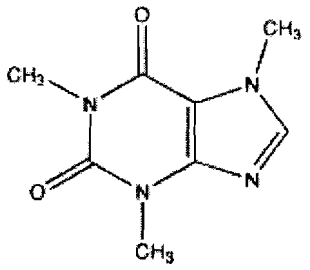
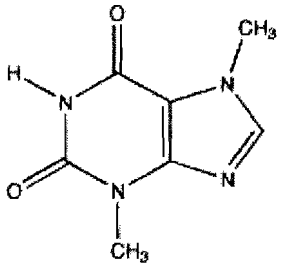


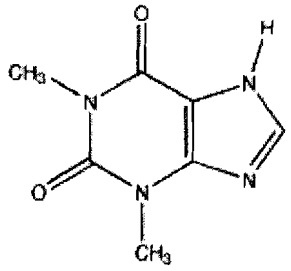
Figure 10. (-)- Epigallocatechin gallate (EGCG)



Caffeine (Caf)



Theobromine (Tb)



Theophylline (Tp)

Figure 11. Caffeine, theobromine and theophylline structures

Mechanism

Green tea is known for its health benefits due the free radical scavenging properties of the antioxidants present. Disturbances in the structure and function of the human cells are caused by the reaction of the oxygen free radicals with vital cellular components such as membranes, nucleic acids and proteins (Xin, Shi, Zhao & Hou, 1995). Excessive oxygen free radicals could damage or kill normal cells resulting heart diseases, tumors or other serious conditions. These diseases could be cured or prevented by scavengers of active oxygen radicals. Such scavenger are in green tea, which is known for its beneficial pharmacological and physiological effects, like

antihepatotoxic (prevents against hepatitis B), antipyretic (fever preventing properties), diurectic (helps in reducing water in the body) and antioxidative properties. If the free radicals are not removed by scavenging them then biological damages could result from lipid peroxidation, which is involved with a series of free-radical-mediated chain reaction processes (Chen, Zhang & Xie, 2005).

Epimerization of Catechins During Processing

During the manufacturing and brewing of green tea, tea catechins undergo chemical changes such as oxidation and epimerization reactions (changing of configuration of a compound into another by enzymatic actions) (Wang & Helliwell, 2000). Steps of steaming or firing are generally used to inactivate the enzymes thus limiting the oxidative reactions. The change in tea catechins by epimerization has been studied using both tap and purified water. The study by Wang and Helliwell (2000) show that epimerization occurs easily in tap water when compared to the purified water. The main factor influencing the difference in tap and purified water are complexity of ions and the pH of the water. This study also showed that not only the temperature but also heating time influences the epimerization of tea catechins. Use of purified water in this research study limited the changes in tea catechins that might have occurred due to the epimerization reactions.

Decaffeination Process of Green Tea

Green tea is composed of various components including catechins, methylxanthines and essential oil compounds (Seok Park et al., 2007). The composition of components varies with the strain of tea tree, the manufacturing process and the harvest time. Catechins are the primary bioactive components of green tea. Catechins are known for their biological and physiological benefits such as anti-inflammation, anti-oxidative, anti-aging, antiviral and antibiotic effects.

Among catechins, EGCG is known for its profound antioxidative property. Green tea health benefits and purported cancer preventive activities have been extended to clinical treatments (Fujiki, Suganuma & Miyazaki, 2005). Green tea extract and EGCG have been found to target the following organs such as digestive (stomach, esophagus, liver, duodenum and colon), plus cancer, bladder, prostate, breast and skin; and to inhibit the growth of cancer cells. Due to the health benefits associated with the catechins in green tea, consumption of green tea and its inclusion into various products are increasing including drinks, ice cream, beauty items and cosmetics (Seok Park et al., 2007). Conversely, the component of green tea, caffeine, is shown to exert negative effects in humans such as sleep deprivation, and hypersensitivity. Daily intake of less than 300 milligrams (mg) of caffeine is considered to be a safe level according to American Beverage Association and the International Food Information Council. Many efforts have been made to remove caffeine from caffeine-containing foods such as coffee, soda and tea. The traditional decaffeination process with the use of organic chlorinated solvents such as trichloroethylene or methylene chloride is receiving growing dissatisfaction from consumers. Some chlorinated solvents have been banned due to probable causing cancer effects in humans as warned by the National Cancer Institute (Seok Park et al., 2007). For the decaffeination process of coffee, water extraction and supercritical carbon dioxide have been commercially employed. The quality of the decaffeinated product is influenced by the amount of the isolated caffeine, any solvent residue and the remaining catechins.

In additional to influencing catechins, the decaffeination process may also have an impact on the other components of green tea (Seok Park et al., 2007). To avoid this impact, selective processes for the removal of caffeine have been attempted by using hot water and microbial degradation of caffeine. Another method is to develop genetically modified (GM) coffee plants

without synergizing the caffeine. These methods are found to have some limitations. One such limitation is that the method using hot water for example, is only applicable to green tea in the fresh form.

An additional technique, supercritical carbon dioxide (SC-CO_2), has several positive effects and is reported to be an ideal non-polar extraction solvent (Seok Park et al., 2007). The positive features of SC-CO_2 are high diffusivity, high dissolving power and low viscosity. It is easily recovered and safe for humans. The removal of caffeine was less effective using SC-CO_2 than when using the Soxhlet method since SC-CO_2 proved to be not feasible.

Super critical CO_2 is considered to be non-polar solvent because of the fact that CO_2 has two oxygen atoms attached in perfect symmetry to a carbon atom (Seok Park et al., 2007). A polar solvent is desirable to be added to SC-CO_2 to increase the polarity and extract the high polar caffeine from the green tea. In the study conducted by Seok Park and colleagues (2007) water and ethanol (95%, v/v) were used as co-solvents to increase the polarity of the catechins. The catechins were analyzed using HPLC and the most abundant component found in decaffeinated green tea composition was EGCG. The order of the catechins were found to be EGCG>EGC>ECG>EC. This study indicates that the decaffeination process using supercritical extraction technique (Figure 11) reduced the caffeine to 2.6% of the initial content. There was also substantial loss of EGCG up to 37.8%, which was unavoidable using this process.

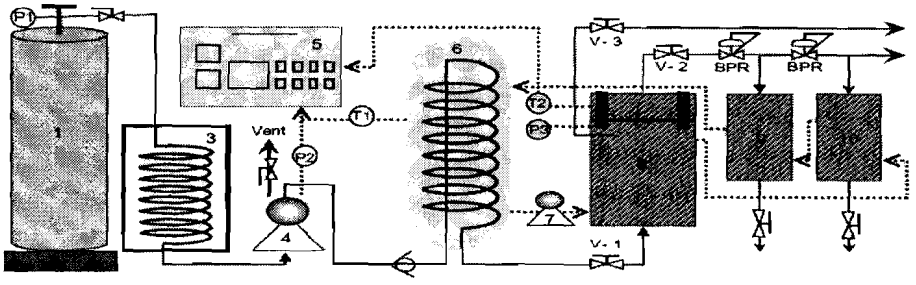


Figure 12. Decaffeination process of green tea (Seok Park et al., 2007)

The decaffeination technique of hot water was used because it leaves no residue and is an inexpensive method (Liang et al., 2007). The concentration of caffeine decreased from 23.7mg/g to 4.0 mg/g when the tea leaf to water ratio of 1:20 (w/v) was used during water extraction. This process was able to remove 83% of caffeine while retaining 95% of the total polyphenols. However, this process was not applicable to black tea as high amounts of catechins were lost if rolled and dried tea leaves are used instead of fresh leaves as in case of green tea.

EGCG Extraction

EGCG can be extracted by a simple method of boiling water and steeping for ten minutes that does not require any expensive equipment (Copeland, Clifford & Williams, 1998). The first step involved decaffeination of tea that was brewed with water and allowed to cool until the temperature reached 60-70 °C. The cooled brew was then divided with a separatory funnel by shaking for 1 minute with an equal volume of chloroform. After settling, the lower layer composed of chloroform and caffeine, was discarded which decaffeinated the teas, and the upper layer of settled mixture was extracted similarly for three times (Copeland et al., 1998). The extraction of flavanols typically followed a similar method, but the chloroform was replaced by a range of solvents in the ratio 3:5 v/v. The organic layers formed after shaking and settling were evaporated to dryness and any residues left was re-dissolved in a minimal volume of water. The decaffeinated green tea brew was purified further by solvent precipitation with propyl acetate and ethyl hexanoate. From this general technique, 25 grams of a commercial green tea leaf yielded 400 mg of (-) EGCG which was better than 80% purity.

Instrumentation for Quantification of Polyphenols

Following the above mentioned extraction, several methods have been used for analysis of polyphenols. Analysis by electrophoresis is faster, but is 1/5 as sensitive as High Performance

Liquid Chromatography (HPLC) (Friedman et al., 2005). To determine the content of catechins and caffeine in black, green, and fruit teas consumed in United Kindom, Khokhar and Magnusdottir (2002) used HPLC with acetonitrile as the eluent (as cited in Friedman et al., 2005). The HPLC method with a photo diode array detector was used by Cabrera and others (2003) to measure four catechin and caffeine levels in 15 green, oolong and black teas sold in Spain (as cited in Friedman et al., 2005).The solvent used for the extraction was 80% methanol for three hours and then twice more at 80% methanol containing 0.15% HCl for three more hours. Another analysis was conducted on Indonesian green and black teas using HPLC-MS to measure the catechins, theaflavins and purine alkaloids by extraction with boiling water for 3 minutes by Del Rio and others in 2004 (as cited in Friedman et al., 2005). Extraction of polyphenols from vegetables, fruits and teas with 90% methanol and 0.5% acetic acid was also conducted by Sakakibara and others in 2003 (as cited in Friedman et al., 2005).

Another study on the use of the instrumentations for quantification of catechins was conducted to investigate the use of the HPLC with ultraviolet and MS (mass spectroscopy) detection for the quantification of green tea catechins (Pelillio et al., 2004). In this study, reversed phase HPLC was used for the analysis of a green tea extract. Comparing the results from both the detection system, similar precision results on the quantifications of green tea catechins was observed, but HPLC with MS system shown less accurate results and provided less detector response.

Studies indicate the use of different instrumentation for the quantification of green tea catechins; HPLC is found to be the most commonly used instrumentation. Separation of various catechins is usually conducted by HPLC having an internal column of 4.6 mm or higher (Pellilo et al., 2004). A high sensitivity level is provided by an ultraviolet detector and is considered to be

suitable for the analysis of catechins. The disadvantage of the ultraviolet detector is that it does not differentiate the compounds with similar chromophore groups. A mass spectrometer is a powerful tool for analysis (identification and confirmation of molecular structure) of unknown compounds that will differentiate the compounds based on the structures.

HPLC is commonly used for the analysis of catechins of green tea. Other techniques and instruments for analysis of catechins include a study that was one conducted on High Performance Capillary Electrophoresis (HPCE) (Bonoli et al., 2003). The features of HPCE are short time analysis, small sample volumes, sensitivity, low running costs and an aqueous mobile phase. This method is in the development stage and many studies have been done regarding the application of this method for the analysis of catechins in teas.

Reverse phase HPLC with UV detection having an isocratic elution system (methanol/water/phosphoric acid) was used for analysis of catechins (Wang, Helliwell & You, 2000). This system provided rapid analysis and accurate results and was found be useful to detect low concentrations of catechin and EGCG. Caffeine and gallic acid was also separated using this system. The column used was a C18 reversed phase Kingsorb 5 μ (150x4.6 mm) with a flow rate of 1.0 mL/min (Wang et al., 2000). The column was operated at 30 °C. The injection volume of sample was 20 μL. Two wave lengths 210 nm and 280 nm were tested in this study and the absorption wavelength was selected at 210 nm. Further research can be conducted to study the quantification of polyphenols using an isocratic elution system.

Figure 12 shows the chromatograms of the effect of orthophosphoric acid in the mobile phase on the separation of analytes. Where, a: a mobile phase consisted of methanol/water (20/80) without orthophosphoric acid; b: a mobile consisted of methanol/water/orthophosphoric acid (20/79.9/0.1); c: a mobile phase consisted of acetonitrile/water (10/90) without

orthophosphoric acid; d: a mobile phase consisted of acetonitrile/water/orthophosphoric acid (10/89.9/0.1) and 1: Gallic acid; 2(+)-GC; 3(-)-EGC; 4:(+)-C; 5: Caffeine; 6: (-)-EGCG; 7: (-)-EC,8(-)-GCG;9:(-)-ECG (Wang et al., 2000). Chromatogram b shows complete separation with orthophosphoric acid added to methanol/water system. Chromatogram a (methanol /water system without orthophosphoric acid) shows poor separation. The gallic acid and (+)-GC were not separated. Also in chromatogram c (acetonitrile/water system without orthophosphoric acid) shows poor separation of gallic acid and (+)-GC and (-)-EGC could not be separated from (+)-C. Chromatogram d was well separated but (-)-EGC was not separated from (+)-C. Therefore, adding orthophosphoric acid to the methanol/water system is shown to provide good separation of the compounds and could use this solution for further research on the quantification of polyphenols.

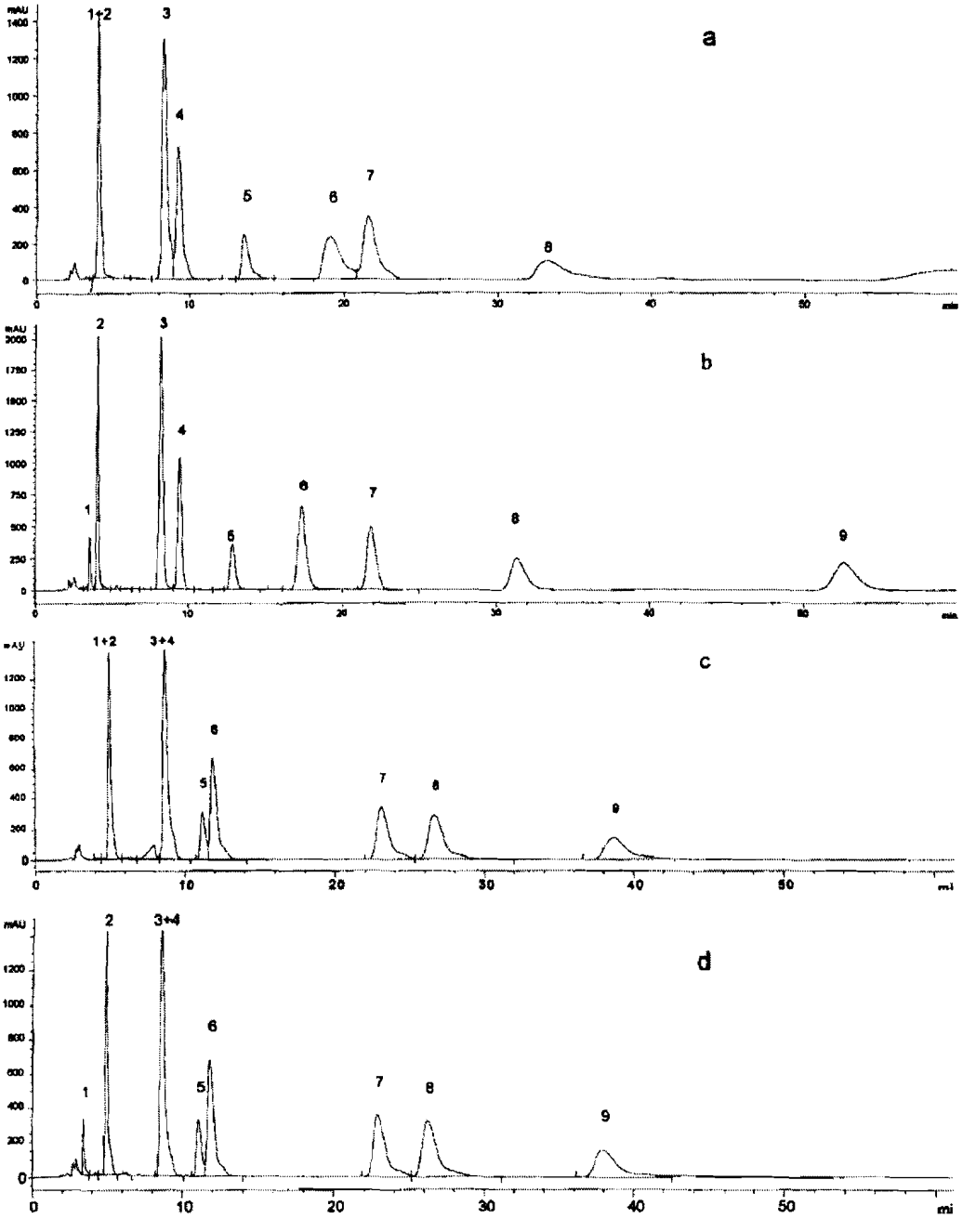


Figure 13. The effect of orthophosphoric acid in the mobile phase on the separation of catechin, caffeine, and gallic acid. Column: 5 microns (μ) C18 (150x4.6 mm); Detection: 210 mm; Flow rate 1.0 mL/min.

(Wang, Helliwell & You, 2000)

Health Benefits of Green Tea

One benefit of consuming green tea is that carcinogenesis in the digestive tract is postulated to be inhibited by ECGC as demonstrated in cells (Okabe et al., 1999). This study showed that polyphenols from tea inhibited the growth and disintegration of a human stomach cancer cell line KATO III, and also inhibited tumor necrosis factor-α (TNF-α) release from the cells. The order of polyphenols that followed the inhibition was ECG, EGCG, EGC and theaflavins. Inhibition of TNF –α release from a human stomach cancer cell line, KATO III, took place with the tea polyphenols (ECG, EGCG, EGC) treated with okadiac acid. EGCG was shown to prevent neuronal cell death caused by several neurotoxins (Reznichenko et al., 2005). Gastrointestinal tract cancer is mainly associated with an excess intake of protein and fat (Borrelli, Capasso, Russo & Ernst, 2004). The polyphenols of green tea have been shown to exhibit inhibitory effects on cancer of the gastrointestinal tract and also preventive effects against several other types of cancer. The evidence to support the preventive effects of green tea polyphenols on stomach and intestinal cancer is not clear. Studies indicate that green tea has a protective effect on adenomatous polyps and chronic atrophic gastritis formations. The inhibitory effect of green tea polyphenols was studied on the human lung cancer cell line, PC-9 (Okabe et al., 1997). Polyphenols that were examined included EGC, ECG, EGCG, and EC. Comparing their inhibitory effect, ECG and EGC showed the same potency as EGCG but EC showed less inhibitory effect. These studies shows the protective effects of green tea and health benefits associated with it on the cell line studied.

Breast carcinoma is considered to be one of the most common cancers in women (Nakachi et al., 1998). Breast cancer is more prevalent in western countries compared to Japan because of their daily intake of green tea as part of the diet. Consumption of green tea prior to the

clinical cancer onset is believed to have decreased the risk of stage I and II breast cancer in women.

Drinking green tea is believed to inhibit certain cancers, such as lung, skin, esophagus, liver, and stomach (Mandel, Weinreb, Amit & Youdim, 2004). Tea catechins are mainly absorbed by the small intestine and are metabolized by enzymatic reactions. Epidemiological studies on the consumption of green tea and risks associated with cancer are still not clear. The reasons for the inconclusive studies might be due to poorly designed studies, lifestyle, and differences in metabolic systems of individuals.

An additional health benefit associated with EGCG is the inhibition of the tumor invasion and angiogenesis processes that are found to be essential for the growth of tumors and subsequent metastasis (Jung & Ellis, 2001). One of the reasons that green tea is of growing interest is the epidemiological evidence indicating a lower risk of various cancers by consumption of green tea and hence EGCG. The biological activities of green tea polyphenols are mainly attributed by the anticarcinogenic and antiproliferative effects of consuming green tea.

Non-melanoma skin cancer is considered to be the most common malignancy in humans (Yusuf, Irby, Katiyar & Elments, 2007). Over 1.3 million cases have been reported in United States. Polyphenols of green tea have been observed to have chemopreventive effects and also inhibitive effects such as carcinogenic activity against ultraviolet (UV) radiation. Thus, ingesting green tea in conjunction with sunscreen use could potentially protect the skin against adverse effects caused by UV radiation.

Along with the antioxidative activity, green tea is shown to exhibit tumor suppressing activity in a cell (Li et al., 2000). For example, polyphenols of green tea were added to human

gastric cancer cells with induced apoptosis, and other tumor cells, and on mouse skin tumorigenesis. The possible health benefits are due to the respond to the negative effects of free radicals by utilizing defensive antioxidants present in green tea.

Intestinal digestion. Although more information is available on the health benefits of green and black tea, little is known about the tea components in the gastrointestinal tract (Record & Lane, 2001). Changes in tea catechins occur with the changes in pH as is found in the gastrointestinal tract. Tea was incubated at an acidic pH, similar to the pH in the stomach. Little effect was observed on the concentration of tea catechins whereas the tea incubated at alkaline pH (similar to small intestine) showed decline in the concentration of both green and black tea catechins (Record & Lane, 2001). This study shows that incubations of green teas at alkaline pH results in decrease in concentrations of catechins, and incubation at higher pH shows less effect on catechins.

Anti-microbial activity. Polyphenols of green tea are known for their biological activities like inhibition of allergies, inhibition of tooth decay, inhibition of oxidation, and reduction of blood pressure (An et al., 2004). The antioxidant level of green tea was more effective on lipid oxidation than the synthetic antioxidant, butylated hydroxytolune (BHT). One study on irradiation used green tea polyphenols that were irradiated with dose of 40 Kilogrey's (kGy) more than the mass of the recommended level by the International Consultative Group of Food Irradiation (ICGFI) as a safe dose for better understanding the effect of irradiation. Irradiation of polyphenols increased the anti-microbial activities against *Staphylococcus aureus* and *Streptococcus mutans*. The study indicated that irradiation of green tea polyphenols maintains the biological activities and increases the anti-microbial activities (An et al., 2004). This study shows the effect of irradiation on changes of biological and anti microbial activities

by irradiating the polyphenols of green tea samples and comparing with non-irradiated samples. From the results of this study, the inhibition ranges for various bacterial (*Escherichia coli, S. aureus, S. mutans)* were 9.3, 10.1 and 9.3 mm in non-irradiated control but 10.8, 11.0 and 11.7 mm in irradiated samples, respectively.

A study was conducted to evaluate the inhibitory effects of grape seed extract, green tea extract, nisin and their combinations against *Listeria monocytogenes* (Theivendran, Hettiarachchy & Johnson, 2006). The inhibitory effect was evaluated in a phosphate buffer solution (PBS) medium which contains 10^9 colony forming units (CFU) of *L. monocytogenes.* Turkey frankfurters were inoculated by 10^6 CFU/g of *L. monocytogenes.* The inoculated frankfurters with and without the addition of antimicrobial agents were dipped in soy protein forming soy solutions and stored at 4 °C or 10 °C (Theivendran et al., 2006). Weekly for 28 days, the inhibitory effects of edible coating were evaluated for frankfurters. Growth and recontamination of *L. monocytogenes* is shown to be controlled by an edible fat coating containing either grape seed extract or green tea extract in combination with nisin in ready-to-eat meat products.

Green tea is known for its purported chemopreventive and therapeutic properties. Tea is also known for its reported antimutagenic, anticarcinogenic, and anticlastogenic effects (Perva-Uzunalic et al, 2006) and for its cancer preventive properties in Japan (Fujiki et al., 1997). EGCG and green tea are of growing interest for cancer prevention due to their important features such as having no toxicity from consumption, and having a wide range of target organs and inhibition effects on the growth of cancer cells (Okabe et al., 1997). One study shows that three components of green tea, namely epicatechin gallate (ECG), epigallocatechin gallate (EGCG), and epigallocatechin (EGC), inhibited the growth of PC-9 cells, while epicatechin (EC) did not

show significant growth inhibition. These results are shown to provide new insights for EGCG and green tea extracts for mechanism of actions against cancer.

Cancer

In the United States, heart disease was considered the first principle cause of death and cancer as the second cause (Bushman, 1998).

Pancreatic cancer. In one of case control studies completed by Jo and coworkers, the risk of cancer was decreased significantly in women by continuous consumption of green tea (as cited in Bushman, 1998). Of these case control studies completed, two case control studies indicated an inverse association and one indicated a positive association between the consumption of green tea and its effect on cancer. Increased risk was associated with higher green tea consumption ($\geq$ 5 cups/day). There is scope for more research on green tea and its association with pancreatic cancer due to preliminary findings.

Colorectal cancer. Five case studies were considered and out of the five studies, three found an inverse association of green tea and colon cancer and one found the positive association (Bushman, 1998). In cases of rectal cancer, only one study showed a statistically significant inverse association out of four studies.

Stomach cancer. Many studies have been conducted to find the association between green tea and stomach cancer (Bushman, 1998). Of these studies, one ecological and four case-control studies show that there is an inverse association of green tea with stomach cancer. Epidemiological studies indicate that drinking five cups or more of green tea results in a lower risk of stomach and esophagus cancer (Weisburger, 1996). Tea prevents the formation of nitrosamide (nitroso compounds) caused by the reaction of nitrates with suitable substrates which can induce cancer.

Lung cancer. Drinking green tea is shown to lower the risk of lung cancer (Weisburger, 1996). Use of tobacco can cause cancer in the oral cavity, lungs, pancreas, kidneys, bladder, esophagus, and cervix. People in Japan smoke more than Westerners but the incidence of lung cancer is lower in Japan which is attributed to their tradition of drinking tea.

Polyphenolic constituents in varieties of teas. Cellular antioxidants are categorized into three classes (Dreosti, n.d). One is endogenously synthesized compounds with antioxidant activity, for example uric acid, glutathione, and lipoic acid. The second category is externally divided antioxidant nutrients like vitamin A, C and E, and beta-carotene. The third category is externally divided non-nutrient antioxidants like polyphenols, salicylates, and bioflavonoids.

To be more focused on the polyphenols which are non-nutrient antioxidants, it is shown that the difference in the relative levels of epicatechins and their oxidized condensation products makes the green tea differentiable from black tea (Table 1) (Dreosti, n.d).

Table 1

Polyphenolic Constituents in Green and Black Tea Beverages (%Dry Solids)

Constituent	Green Tea (%)	Black Tea (%)
Flavanols	30-40	5-10
Epigallocatechin gallate	10-15	4-5
Epicatecchin gallate	3-10	3-4
Epigallocatechin	3-10	1-2
Epicatechin	1-5	1-2

(Dreosti, (n.d.).)

A study was conducted to investigate the concentrations of polyphenols extracted using multi step extraction process (Perva-Uzanalic et al., 2006). Table 2 shows the amount of catechins extracted using multi step extraction at time of 95 °C and 10 minutes. The multi-step extraction consisted of four steps, where after extraction; the residue left is extracted using water at 95 °C and 10 minutes. This process is repeated similarly for steps three and four. The results show that the extraction for multiple times decreased the EGC and EC contents but the EGCG increased.

Table 2

Multi-step Extraction of Green Tea with Water at Ratio 40 mL: 1 g (T_s=95°C, t=10 minutes)

Extraction Step	EGC (g/Kg dry extract	ECG (g/Kg dry extract)	EC (g/Kg dry extract)	EGCG (g/Kg dry extract)	Caf (g/Kg dry extract)
1	126	47.8	1.2	239	71.4
2	108	59.0	1.1	243	75.5
3	68.5	91.6	1.1	420	54.5
4	42.3	127	0.1	484	34.0

(Perva-Uzanalic et al., 2006)

Under the same study conducted by Perva-Uzanalic and others in 2006, the concentration of polyphenols and caffeine was analyzed in the starting material of green tea. Table 3 shows amount of catechins and caffeine in green tea leaves of varieties Fanning Belas. The concentrations of major catechins and caffeine were 191 g/Kg material; and 36.0 g/Kg material, respectively. Among the catechins analyzed, EGCG represents 67.5% of the major catechins in green tea leaves.

Table 3

Amount of Catechins and Caffeine in Green Tea Leaves, Variety Fanning Belas

Active ingredient	Content (g/Kg dry leaves)	Content (g/Kg dry leaves)
Caffeine		36.0
Catechins		
Epicatechingallate		15.2
Epigallocatechin		46.0
Epigallocatechin gallate		129
Epicatechin		0.90

(Perva-Uzanalic et al., 2006)

Polyphenols constitute up to 36% in dry green tea leaves and among those polyphenols measured catechins are the most prevalent (Perva-Uzunalic et al., 2006). A study was conducted by Perva-Uzunalic and colleagues in 2006 on the extraction efficiency of major catechins and caffeine using a temperature range from 60 °C to 95 °C and an extraction time of 1 to 120 minutes without maintaining constant temperature. This study aimed to look for the concentrations of catechins at various temperatures for a long extraction time. One-hundred milligrams of distilled water was poured on 1 gram of green tea leaves. The temperature decreased from 95 °C over the period of time (1 to 120 minutes) and the temperature was noted. Optimum conditions were determined for the extraction procedure with water (Perva-Uzunalic et al., 2006). The extraction efficiency of catechins with water maximized at 80 °C after 20 minutes (97%) and at 95 °C after 10 minutes (90%). In general, the extraction efficiency and quality of the extract from tea is usually influenced by the conditions including tea, solvent type, time,

temperature, ratio of solvent to material and pH. Higher temperatures for a shorter period of extractions might provide maximum extraction efficiency.

Another study was conducted by Wang and Helliwell (2000) to demonstrate the effect of different water types (tap and purified water) on the extraction of the polyphenols in green teas. The process includes, brewing gunpowder tea infusions using purified water and tap water and then cooling immediately to ambient temperature. After cooling the infusions were heated at 100 °C for variety of times, 0 hour, 20 minutes, 1 hour, 3 hour and 5 hours (Table 4). From the results, the conversion from (-)-EGC and (-)-EGCG to their corresponding epimers, (-)-GC and (-)-GCG, in the tea brewed using purified water increased during the first three hours and then gradually decreased (Wang & Helliwell, 2000). However, (-)-EC and (-)-ECG, and their corresponding epimers increased continuously during the five hours heating. In tap water, (-)-GC, (-)-GCG and (-)-CG reached a higher level after 20 minutes of heating, and (-)-C reached a higher level after 1 hour of heating. This state of stabilization indicates that the catechins after reaching the maximum level of epimerization, the most change become the degradation or oxidation of the catechins. This study also concluded that catechins were found to be highly unstable in the infusions of tap water.

Based on the findings, using purified water for the present research possibly have provided better results than using the tap water.

Table 4

Contents of Catechins in Green Tea Infusion when Heated at 100 °C (mg/100 mL)

Compound	0hr	20 min	1 hr	3 hr	5 hr
Purified water					
(-)-EGC	19.0	14.6	12.6	8.40	5.34
(-)-GC + (+)-GC	1.45	2.94	3.61	5.66	5.36
(-)-EGCG	24.2	19.3	17.4	12.1	8.44
(-)-GCG	0.61	2.63	3.58	6.34	6.03
(-)-EC	4.34	3.99	3.87	2.90	2.35
(-)-C+(+)-C	0.51	0.93	1.03	1.71	1.88
(-)-ECG	5.02	4.65	4.52	3.38	2.73
(-)-CG	0.06	0.36	0.43	0.99	1.14
Tap water					
(-)-EGC	9.68	0.28	0.26	0.16	0.15
(-)-GC + (+)-GC	2.00	3.23	1.32	0.16	0.09
(-)-EGCG	13.2	1.34	0.61	0.23	0.15
(-)-GCG	0.98	2.44	1.05	0.34	0.16
(-)-EC	4.05	1.77	1.40	1.04	0.82
(-)-C+(+)-C	0.56	2.87	2.88	2.16	1.77
(-)-ECG	4.06	1.98	1.51	1.02	0.67
(-)-CG	0.09	1.44	1.34	0.95	0.66

(Wang & Helliwell, 2000)

Extraction Efficiency of Catechins and Caffeine

Many studies indicate that the use of different times and temperature conditions for extraction of green tea can impact the catechin levels removed, as well as influence by using different solvents including even water (Friedman, Levin, Choi, Kozukue & Kozukue, 2006). For example, the use of boiling water for 3, 5, 10 minutes, at 80 °C for 30 minutes, at 90 °C for 30 minutes were used as extraction conditions. The use of acetonitrile and water for 1 hour, use of ethanol at room temperature, and the use of acetone for two weeks was also shown to be used as extraction conditions for polyphenols by other researchers. The results showed that the amount of catechins and caffeine extracted in the boiling water for five minutes increased in the temperature range of 60 to 100 °C and was in large amounts at 100 °C for 5 minutes (Friedman et al., 2006). The total range of catechins in green tea was from 51.5 to 84.3 mg/g. The total estimated dietary intake of catechin from green was 40.5.g mg/d and caffeine was from 92 to 146 mg/d. Based on the dietary intake, one to one and half grams of tea leaves could provide the required level of catechins per day.

A study by Friedman and others in 2006 compared the tea constituents extracted using water and 80% ethanol/water in boiling water for 5 minutes and at 60 °C for 15 minutes to determine the amount of polyphenols plus theaflavins. The caffeine content (in mg/g of tea) for green teas extracted with 80% ethanol was in the range from 0.5 to 26.8 and the concentrations extracted with water was in range from 0 to 23.1. The amount of polyphenols extracted using aqueous ethanol was substantially greater than when using water alone.

A study was conducted to investigate the changes in the concentrations of polyphenols if tea was prepared by either adding milk or vitamin C, which is most commonly used method (Majchrzak, Mitter & Elmadfa, 2004). The results showed that adding ascorbic acid (vitamin C)

to green tea increased the total antioxidant activity in green tea extracts linearly up to 30 mg ascorbic acid/100 mL tea solution.

Green Tea Applications

Milk-tea beverages. Tea catechins health benefits are not only associated with antioxidant activities but also include free metal chelation, scavenging of reactive oxygen and nitrogen species, as well as inhibition of lipoxygenase and cycloxygenase (oxidative enzymes) (Ferruzzi & Green, 2006). Knowing the health benefits of tea catechins, new products have been developed with tea as an active ingredient in products such as ready-to-drink (RTD) tea beverages, confections, ice creams, cereal bars and pet foods. According to Ferruzzi and Green (2006) in many countries, tea is formulated with milk in different proportions to improve sensory properties; however, green tea is not traditionally consumed with milk even though formulations of green tea with soy and other dairy products are increasing. Many methods have been developed for effective extraction of catechins in complex food formulations but methods were not suitable for milk based products. Strong catechin-protein interaction occurs in milk based products. Proteins have to be removed by a deproteinization step prior to the analysis so that this step can prevent precipitation onto the HPLC column during analysis. Various methods of deproteinization like pepsin treatment, methanol deprotenization and acid precipitation were studied. Total catechins were highly recovered by pepsin treatment (89-102%) then by methanol deproteinization (78-87%) and followed by acid precipitation (20-74%) (Ferruzzi & Green, 2006).

Studies show that consumption of tea with milk may have less availability of polyphenols, however, other studies have indicated that the addition of milk to black tea does not influence the absorption of tea catechins and its antioxidant activities (Ferruzzi & Green, 2006).

It is a common habit to add lemon to tea in United Kingdom, so a study was conducted to see the effect of lemon on the polyphenols of green tea. The study showed that the addition of lemon (ascorbic acid) increased the total antioxidant capacity linearly up to 30 mg ascorbic acid/100 mL tea solution.

Based on the above mentioned studies, it can be concluded that due to the purported health benefits of green tea, its usage and applications have increased to large extent. The above studies highlight the extensive health benefits of green tea. The information on the effect of extraction parameters on commercial caffeinated and decaffeinated green teas of United States is however, limited. This current study could therefore provide consumers information on the concentrations of polyphenols present in green teas analyzed; as this study is focused on the effect of extraction parameters time and temperature on the polyphenols and methylxanthines of green teas commercially available in United States.

Chapter III: Methodology

The purpose of the study was to quantify the concentrations of catechins (catechin and epicatechin) and their gallic acid analogs (epigallocatechin, epicatechin gallate, epigallocatechin gallate) and the methylxanthines (caffeine, theobromine, and theophylline) as a function of extraction time and temperature.

Reverse phase gradient HPLC was used for analysis and to quantify the concentrations of catechins and their gallate compounds and methylxanthines in three commercial brands of green tea in both caffeinated and decaffeinated forms.

Figure 14. Commercial green teas used for the study

Subject Selection and Description

Three commercial brands of green teas produced in the United States were chosen. The chosen brands were caffeinated and decaffeinated Salada®, Bigelow®, and Celestial Seasonings® green teas (Figure 13). The tea bag boxes of these three brands were purchased from a local grocery store (Menomonie, Wisconsin, USA).

In order to determine steeping conditions, the factory recommended brewing time and temperature conditions were used for these three brands, which are summarized in Table 5.

Table 5

Brewing Conditions for Different Brands of Green Tea

Tea samples	Time (minutes)	Temperature (°C)
Salada®	2-3	77
Bigelow®	3-5	85
Celestial Seasonings®	3	100

Reagents

The solid compounds used to prepare the standards were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). (+)-Catechin Hydrate ≥ 98% purity was purchased in 5.0 gram quantities (catalog# C1251-5G), (-)-Epicatechin ≥ 90% purity was purchased in 1.0 gram quantities (catalog # E1753-1G), (-)-Epigallocatechin ≥ 95% purity was purchased in 5.0 milligram quantities (catalog # E3768-5MG), (-)-Epicatechin gallate ≥ 98% purity was purchased in 10.0 milligram quantities (catalog # E3893-10MG), and (-)-Epigallocatechin gallate ≥ 95% purity was purchased in 50.0 milligram quantities (catalog# E4143-50MG). HPLC-grade acetonitrile and reagent grade 99% acetic acid were purchased from Fisher Scientific (Fairlawn, New Jersey).

Water used for brewing tea was purified by the Milli –Q® water purification system manufactured by Millipore ® Corporation. A PolyScience® water bath was used to maintain the temperature.

Standards

The mass of solid catechins and methylxanthines was measured to the nearest 0.01 milligram using an analytical balance. Measured amounts (Tables 6 and 7) were added to a 25-mL volumetric flask and diluted to volume with methanol to prepare standard mixtures. Individual standards and three different standard mixtures were prepared to compare and identify the peaks of green tea samples to those of the standards. For example, to prepare the standard mixture 1 (Table 7), 2.55 mg of catechin, 2.66 mg of epicatechin, 0.94 mg of epigallocatechin, 1.70 mg of epigallocatechin gallate, 0.52 mg of caffeine, 0.60 mg of theobromine, and 1.50 mg of theophylline were added to a 25-mL volumetric flask and diluted to mark with methanol. Individual standard solutions were also prepared. Tables 8 and 9 show the concentrations of different standard solutions prepared per mg/L.

Table 6

Individual Solids (mg) Diluted to 25.0 mL to Produce Standard Solutions

Identity of standard solution	Mass of Solid (mg)
EC 1	1.23
EC 2	5.86
EC 3	9.45
EGC 1	1.06
EGC 2	2.66
EGCG 1	1.55
EGCG 2	2.40
EGCG 3	7.56
Tp 1	6.43
Tp 2	14.2
Caf 1	1.04
Caf 2	5.89
Caf 3	12.9

Table 7

Mass (mg) of Individual Solids Diluted to 25.0 mL to Prepare Standard Mixtures

Standards	Mass of solids (mg)							
	C	EC	EGC	ECG	EGCG	Caf	Tb	Tp
Standard Mixture 1	2.55	2.66	0.00	0.94	1.70	0.52	0.60	1.50
Standard Mixture 2	4.26	4.00	0.00	2.00	1.07	2.49	2.41	2.61
Standard Mixture 3	1.39	1.60	0.00	1.73	0.30	1.25	1.93	0.63

Table 8

Concentrations of the Individual Solids (mg/L)

Identity of standard solution	Concentrations of Solids (mg/L)
EC 1	51.5
EC 2	227
EC 3	378
EGC 1	42.2
EGC 2	106
EGCG 1	61.8
EGCG 2	96.0
EGCG 3	302
Tp 1	254
Tp 2	566
Caf 1	41.4
Caf 2	236
Caf 3	517

Table 9

Concentrations of the Individual Solids in Standards Mixtures (mg/L)

Standard Mixture	Concentration (mg/L)							
	C	EC	EGC	ECG	EGCG	Caf	Tb	Tp
Standard Mixture 1	102	106	0.00	38.0	68.0	21.0	24.0	60.2
Standard Mixture 2	170	160	0.00	80.1	42.7	99.5	96.2	105
Standard Mixture 3	55.6	63.9	0.00	69.2	12.1	50.0	77.1	25.2

Solvent Gradient

The chromatographic separation required a binary solvent gradient. Solvent A (99.75% water/ 0.25% acetic acid) was prepared by diluting 5 mL of glacial acetic acid to the mark in 2-L volumetric flask with Milli-Q® water. Solvent B (40% acetonitrile / 60% solvent A) was prepared by measuring 400 mL acetonitrile in a 1000 mL graduated cylinder and filling to the mark with solvent A.

Sample Preparation

The sample preparation procedure consisted of bringing Milli-Q® water to different temperatures (100 °C, 95 °C, 90 °C, 85 °C, 80 °C) in 250-mL beakers and steeping the tea bags in the beakers at constant temperature for different times (2, 4, 6, 8 and 10 minutes). Comparing the brewing temperatures of the chosen green tea brands, the temperature varies from 77-100 °C and the brewing temperatures were chosen based on typical recommended temperatures not to extend beyond 10 minutes for consideration of the oxidation of polyphenols. Each beaker was allowed to cool and a portion transferred to an autosampler vial with filtration through a 0.45 micrometer (μm) syringe-mounted membrane filter. This procedure was carried out in triplicate for each variety and each set of conditions.

The mass of tea leaves in each tea bag was determined to the nearest 0.0001g using an analytical balance. Initial mass of each total tea bag was recorded and tea leaves from the tea bag were transferred into the weighing dish and the mass of the empty tea bag was recorded. The mass of the tea leaves was calculated using equation 1.

$$W_{tea\ leaves} = W_{bag+leaves} - W_{empty\ tea\ bag} \qquad 1$$

The extraction was also carried at room temperature (24 °C) with longer extraction times (1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour and 24 hour) in triplicates. The above time and temperatures were chosen, considering the daily tea drinking habits of people. In many cases, it is the tendency to leave the tea bag for more than an hour. Taking this in consideration, the following research was done to determine and quantify the amount of polyphenols at various extraction times at room temperature. The average mass of the tea leaves from tea bags is shown in Table 10.

Table 10

Average mass (g) of Tea Leaves from the Tea Bags of the Green Teas

Green teas	Average mass (g ±SD[1])
Celestial Seasonings®	1.99±0.06
Celestial Seasonings® decaffeinated	1.64±0.09
Salada®	2.20±0.03
Salada® decaffeinated	1.82±0.03
Bigelow®	1.45±0.04
Bigelow® decaffeinated	1.41±0.06

SD[1] - Standard deviation

Instrumentation

A Waters HPLC used for the analysis of catechins and their gallate compounds and methylxanthines was equipped with 1525 binary pump, 717 plus autosampler, NovaPak C18 radial compression column (10 cm x 8 cm) with a NovaPak GuardPak in an RCM-100 radial compression module, 2996 photodiode array detector and EmPower chromatography software.

Solvent A consisted of 99.7% Milli-Q® water/0.25% acetic acid (v/v). Solvent B consisted of 40% acetonitrile and 60% solution A (v/v). A sample volume of 25 microliter (μL) was injected. During the analysis, solvent composition changed from 100% A to 100% B over a 30-minute run time (Table 11). The gradient was slightly concave (nonlinear), corresponding to curve 8 on the Waters solvent programmer (Figure 14). Acetic acid was necessary to prevent ionization of the polyphenols and resulting peak broadening. At the end of the 30-minute gradient, the solvent was returned to 100% A (1 minute) and the system equilibrated for 5 minutes.

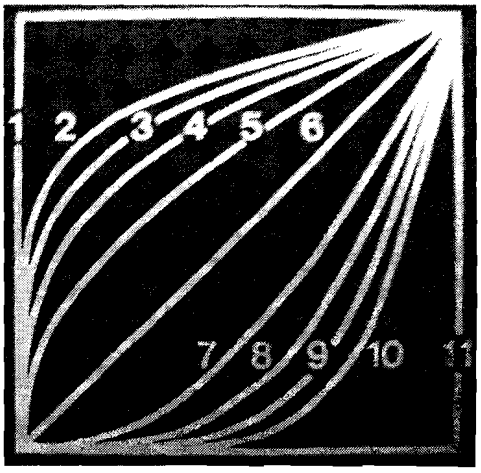


Figure 15. Solvent program curves available for gradient separation

Table 11

Gradient Separation Conditions (Mobile Phase)

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	0.00	2.00	100.0	0.0	0.00
2	30.00	2.00	0.0	100.0	8
3	31.00	2.00	100.0	0.0	6
4	35.00	2.00	100.0	0.0	6

Four wavelengths (260, 270, 280 and 290 nm) were used for detection and peak verification. Multiple wavelength detection is useful for unambiguous peak identification because peak area ratios of unknown peaks can be compared to ratios for standards. If a peak has a retention time similar to that of a known standard, its area must compare favorably. The results from the 270-nm chromatograms are reported in this study because that wavelength is nearest the λmax for the analytes investigated.

An example of the chromatogram for standard mixture #1 is shown in Figure 15, and the Bigelow® green tea chromatograms for an 85 °C at 2 minute extraction and a 100 °C at 10 minute extraction are shown in Figures 17 and 16, respectively. Appendices A through G show the standard plots of the analytes. These plots were used to identify the concentrations of catechin, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, caffeine, theobromine and theophylline in tea samples.

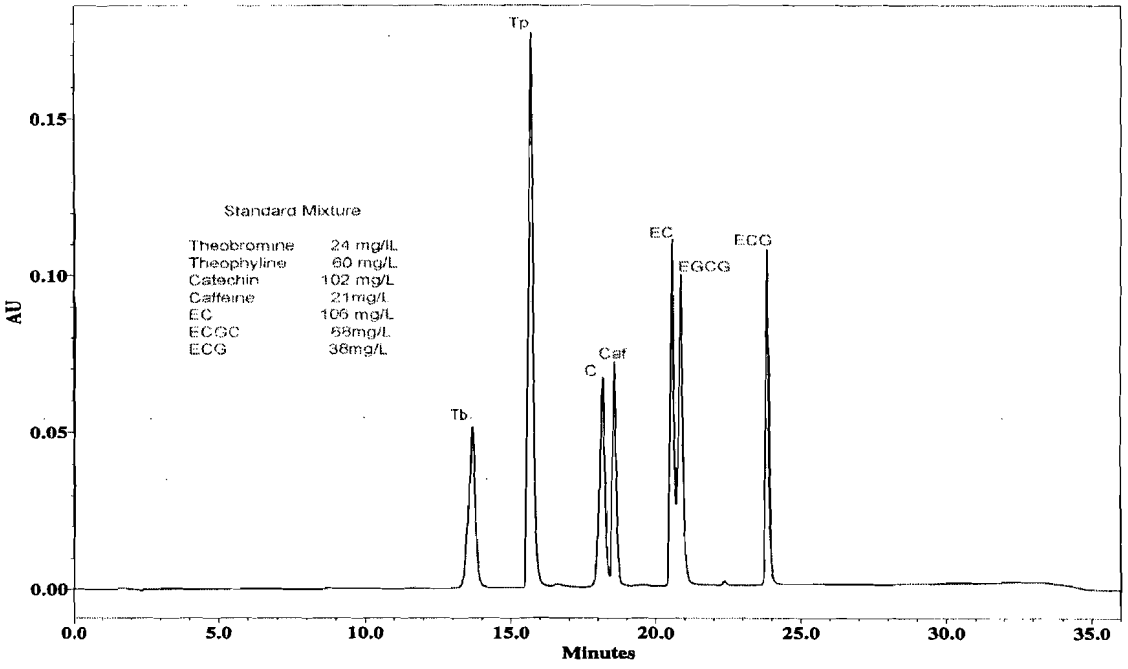


Figure 16. Chromatogram of standard mixture

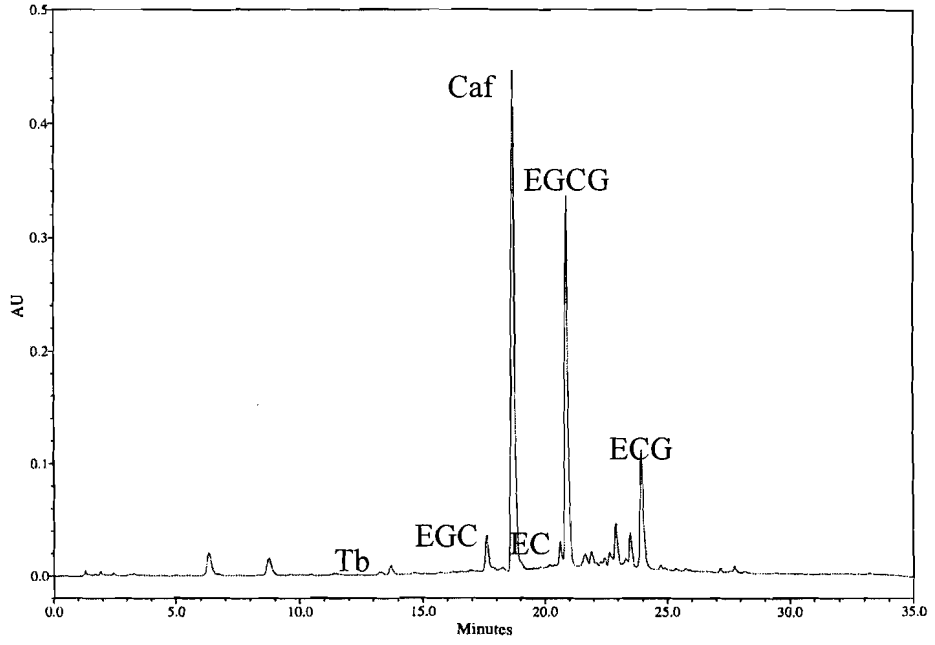


Figure 17. Chromatograph of Bigelow® green tea extracted for 10 minutes with water at 100 °C

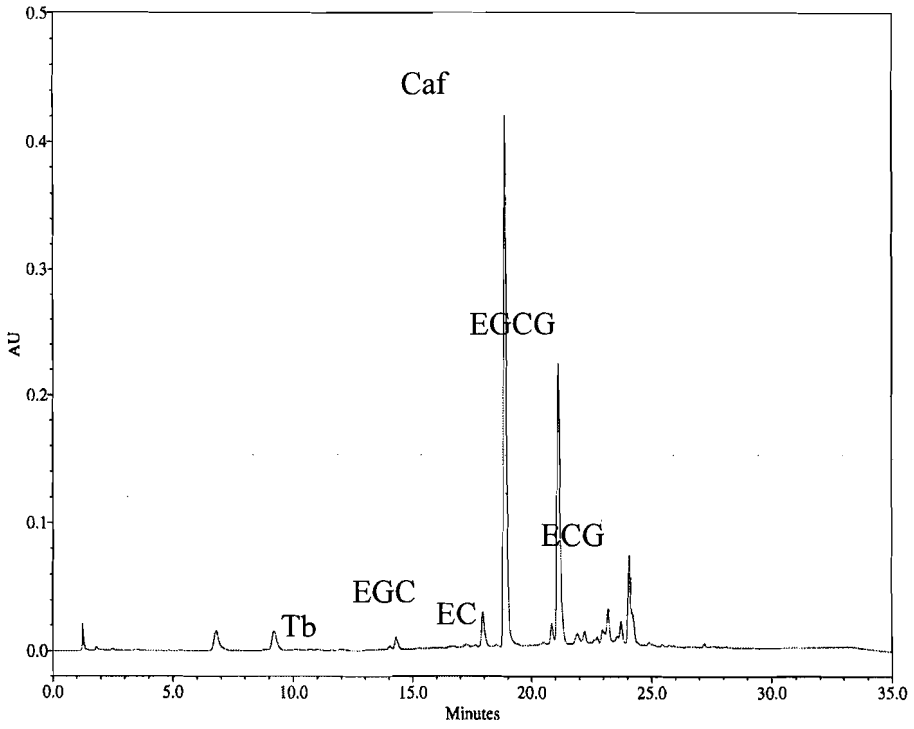


Figure 18. Chromatograph of Bigelow® green tea at 85 °C, 2 minutes

Data Analysis

The mean concentrations and their standard deviations were determined using Microsoft Excel and the graphs were plotted using Logger Pro software.

Using the retention times and peak area ratios of the standards, peaks were identified in all tea samples. The concentration of each analyte in each tea sample was determined from the standard curve by interpolation of the peak area from the 270 nm chromatogram for each sample on the standard curve.

Chapter IV: Results and Discussion

The polyphenols (catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG)) and the methylxanthines (caffeine (Caf), theobromine (Tb), theophylline (Tp)) of green tea were detected and quantified by developing a high performance liquid chromatography (HPLC) method. Commercial green tea brands in the United States, Salada ®, Celestial Seasonings® and Bigelow® both in caffeinated and decaffeinated forms were chosen for the analysis. Extraction conditions with temperatures ranging from 80 °C through 100 °C and times 2, 4, 6, 8 and 10 minutes were chosen, and polyphenols were quantified. Extractions at room temperature (24 °C) for longer periods of time (1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour, and 24 hour) were also investigated.

Analytes were detected at wavelengths of 260 nm, 270 nm, 280 nm, and 290 nm in all HPLC runs. Using the average retention times of the standards and peak area ratios of the standards, peaks for polyphenols and methylxanthines were identified in green tea samples. Peak area ratios calculated for each identified peak were similar to those of the standards. The similar peak area ratios confirmed peak identification of each compound of interest in each tea sample. For each analyte, a standard curve was prepared by plotting concentration (mg/L) on x-axis and peak area (μV*sec) on y-axis using Logger Pro software. Quantification of each analyte was determined by interpolation of its peak area against the corresponding standard graph (Appendixes A-H).

Calculations of the Components

Each component was weighed in grams and converted into milligrams. The obtained concentrations from the standard graphs are in mg/L.

To convert the concentrations of each compound to 250 mL serving size, the following equation was used:

amount of component (mg) x 250 mL /serving size x 1/1000 mL = mg/serving size

The average concentration of the analytes extracted at room temperature (24 °C) for long periods of time (1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour, and 24 hour) from Salada®, Celestial Seasonings®, and Bigelow® green tea samples are displayed in Tables 12, 13 and 14, respectively. All of the concentrations of the compounds are given in mg/serving for ease of comparison with other studies that denote concentrations of the catechins in milligrams per serving. It is also useful to denote how many milligrams of catechins and methylxanthines are present when each tea bag was brewed according to manufacturer's preparation directions (recommends one cup of tea which is equivalent to 250 mL). The concentrations obtained were not compared to other studies as the extraction conditions were found to be different. A study was conducted by Wang and Helliwell (2000) on the effect of time on the epimerization of catechins, where the infusions of tea brewed using both purified and tap water. The tea brewed was cooled to ambient temperature and heated at 100 °C for different periods of time (0 hour, 20 minutes, 1 hour, 2 hour, 3 hour, 4 hour, 5 hour). The results showed that the catechins were highly unstable in the infusions brewed using the tap water. The epimerization increased during the first three hours and then decreased slightly in teas brewed with purified water but for tap water reached a maximum level after 20 minutes.

From Tables 12, 13 and 14, the results show that there was little change in the catechin and methylxanthine concentrations of both caffeinated and decaffeinated forms with the increase of extraction time at a constant temperature (24 °C). A slight decrease in the concentration of polyphenols of both caffeinated and decaffeinated forms was found at 2 hour extraction time

except for EGC in caffeinated Salada® and Bigelow® decaffeinated green teas. The concentrations for most catechin and methylxanthines increased slightly at 3 hours and remained constant except for C and EGC in Celestial Seasonings® and Salada® decaffeinated tea. Generally compounds peaked at 2 to 6 hour, except for EGC in Salada® decaffeinated tea, which was largest in value (7.63 mg/serving) at 24 hour.

Comparing the caffeine concentrations at one hour extraction in three brands of green tea, Salada® caffeinated had the largest concentration of caffeine (40.5 mg/serving) and EGCG (68.9 mg/serving) concentrations, followed by the Celestial Seasonings® caffeine (27.3 mg/serving) and EGCG (28.8 mg/serving) and lowest being Bigelow ® caffeine (19.8 mg/serving) and EGCG (18.8 mg/serving) concentrations (Tables 12, 13, and 14). The other catechins and methylxanthines also followed the same trend. In decaffeinated forms, at 1 hour extraction, Salada® had largest concentration of EGCG (38.5 mg/serving) followed by Bigelow® (34.5 mg/serving) and lastly Celestial Seasonings® (31.4 mg/serving). Looking at the data, it is interesting to note that the Bigelow® decaffeinated green tea had a larger amount of EGCG, nearly 74% compared to the caffeinated form. Celestial Seasonings® decaffeinated tea also has larger amount of EGCG than caffeinated form, but the difference is about 3 mg/serving at 1 hour extraction time. Comparing the catechins, Salada® caffeinated green tea had a larger polyphenol concentration (128 mg/serving) than Celestial Seasonings® (62.7 mg/serving) and Bigelow® (38.4 mg/serving) green tea samples. The variation in the amount of polyphenols depends on the type of green tea leaves used for manufacturing, and the type of extraction method used by each company. Comparing the decaffeinated teas, the Salada® decaffeinated green tea samples had larger amounts of polyphenol (which includes C, EC, EGC, ECG, EGCG) concentrations (80.0 mg/serving) followed by Bigelow® (62.3 mg/serving) and then Celestial Seasonings® (58.9

mg/serving) at 1 hour extraction. Bigelow® decaffeinated green tea had 62% higher amounts of polyphenols than its caffeinated green tea, which is notable. This might be influenced by the decaffeination process used and also the type of green tea leaves. The decaffeination extraction efficiency of Bigelow® green tea is very high compared to Salada® and Celestial Seasonings® green teas since it allowed larger levels of polyphenols to be retained.

The concentrations of caffeine, epicatechin (EC) and epigallocatechin gallate (EGCG) are plotted against the extraction time for Salada® tea (Figure 18). The concentration of caffeine increased through 4 hour extraction and then tended to decrease in concentration. The concentrations of epicatechin and epigallocatechin gallate increased until 4 hour extraction time and remained almost constant until 7 hour extraction and decreased after 24 hour extraction time. Comparing all the caffeinated green teas analyzed, the concentrations of EGCG decreased from 7 hour extraction time to 24 hour extraction time by around 9-11 mg/serving (for example in Salada® tea, 84.8 mg/serving – 73.6 mg/serving = 11.2 mg/serving) in both Salada® and Bigelow® green tea but interestingly the Celestial Seasonings® showed less of a decrease in EGCG (2 mg/serving) compared to all others. The reason for less decrease in concentration could be because of the vitamin C added to it that may have stabilized the polyphenols. A Vitamin C peak was observed at a retention time of around 2 minutes in one of the chromatographs obtained for Celestial Seasonings® run (Figure 19). Epicatechin concentration increased to about 4.5 mg/serving after 1 hour extraction time and remained almost constant until 7 hours but interestingly a huge amount of decline was observed at 24 hour extraction time, which could be either experimental error or the manufacturing process of decaffeination in Celestial Seasonings® tea (Table 13).

Table 12

Concentrations (mg) of Catechins and Methylxanthines per 250 mL Serving in Salada® Green Tea Samples at Room Temperature 24 °C

Green Tea Sample	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	24 hr
Salada caffeinated								
Tp[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tb	1.43	1.29	1.63	1.71	1.63	1.65	1.64	1.50
Caf	40.5	37.2	48.1	50.2	47.8	48.9	48.9	44.8
C	30.7	27.2	31.4	33.6	31.8	33.5	28.0	29.9
EC	11.8	10.4	13.6	13.9	13.7	13.5	13.9	12.3
EGC	3.04	4.36	6.07	6.39	6.31	5.45	3.04	2.49
ECG	13.7	13.1	22.9	22.9	22.7	16.9	17.8	15.6
EGCG	68.9	64.9	84.5	87.2	84.6	84.0	84.8	73.6
Salada								
decaffeinated	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tp[1]	0.88	0.83	0.92	0.66	0.84	0.89	0.91	0.92
Tb	3.43	3.14	3.60	5.40	3.47	3.63	3.63	4.32
Caf	17.9	16.1	15.9	13.7	15.5	14.5	16.7	17.4
C	8.88	5.96	6.53	5.20	6.00	6.34	6.30	7.71
EC	6.93	3.04	1.40	2.02	2.18	2.18	2.41	7.63
EGC	7.81	7.35	8.99	6.32	7.75	8.57	8.90	8.10
ECG	38.5	36.6	42.3	31.5	37.8	40.3	41.9	39.4
EGCG								

[1]n/a=not available

Table 13

Concentrations (mg) of Catechins and Methylxanthines per 250 mL Serving in Celestial Seasonings® (CS) Green Tea Samples at Room Temperature 24 °C

Green Tea Sample	1hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	24 hr
CS caffeinated								
Tp[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tb	0.29	0.50	0.54	0.37	0.40	0.45	0.45	0.43
Caf	27.3	28.4	27.3	24.9	28.3	31.6	31.4	29.3
C	20.3	26.0	30.0	29.5	28.5	29.7	29.5	28.3
EC	5.77	10.3	11.4	9.79	11.2	12.5	11.5	1.06
EGC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ECG	7.81	7.35	8.99	6.32	7.75	8.57	8.90	8.10
EGCG	28.8	41.0	50.9	42.8	49.1	54.9	55.1	53.2
CS Decaffeinated								
Tp[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tb	0.92	1.08	1.18	1.19	1.09	1.05	1.11	1.05
Caf	2.75	4.13	4.59	4.58	4.23	4.17	4.35	4.12
C	13.6	14.6	15.5	15.4	14.0	13.7	15.2	13.8
EC	5.66	6.07	6.61	6.53	6.23	5.88	6.34	6.26
EGC	1.64	1.64	1.56	1.40	1.25	1.09	1.09	1.17
ECG	6.62	9.17	10.5	10.3	9.22	9.41	10.0	9.01
EGCG	31.4	38.9	43.7	43.7	40.5	39.8	41.9	38.0

[1]n/a=not available

Table 14

Concentrations (mg) of Catechins and Methylxanthines per 250 mL Serving in Bigelow® Green Tea Samples at Room Temperature 24 °C

Green Tea Sample	1hr	2hr	3hr	4hr	5hr	6hr	7hr	24hr
Bigelow Caf[2]								
Tp[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tb	0.45	0.32	0.36	0.46	0.41	0.52	0.51	0.49
Caf	19.8	14.6	17.1	22.1	19.8	24.6	24.7	23.7
C	10.6	7.41	8.33	10.3	9.14	10.9	11.1	13.4
EC	5.20	3.53	4.06	5.54	4.90	6.34	6.72	7.40
EGC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ECG	3.82	3.19	3.93	5.36	4.67	5.88	5.93	3.69
EGCG	18.8	14.3	16.9	21.8	19.4	23.8	24.0	15.5
Bigelow Deca[3]								
Tp[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tb	0.82	0.79	1.12	1.00	1.04	1.06	1.06	0.89
Caf	2.25	3.51	3.12	3.81	2.90	3.15	3.17	2.37
C	11.6	14.5	16.4	14.8	15.1	15.5	15.4	11.2
EC	6.23	7.18	8.05	7.18	7.56	7.74	7.67	6.42
EGC	1.79	3.97	2.80	2.57	2.49	2.49	1.01	0.16
ECG	8.21	13.3	15.8	15.5	14.9	16.9	13.0	10.4
EGCG	34.5	41.8	49.1	45.2	46.7	48.1	48.2	39.2

[1]n/a=not available
Caf[2]= caffeinated
Deca[3]=decaffienated

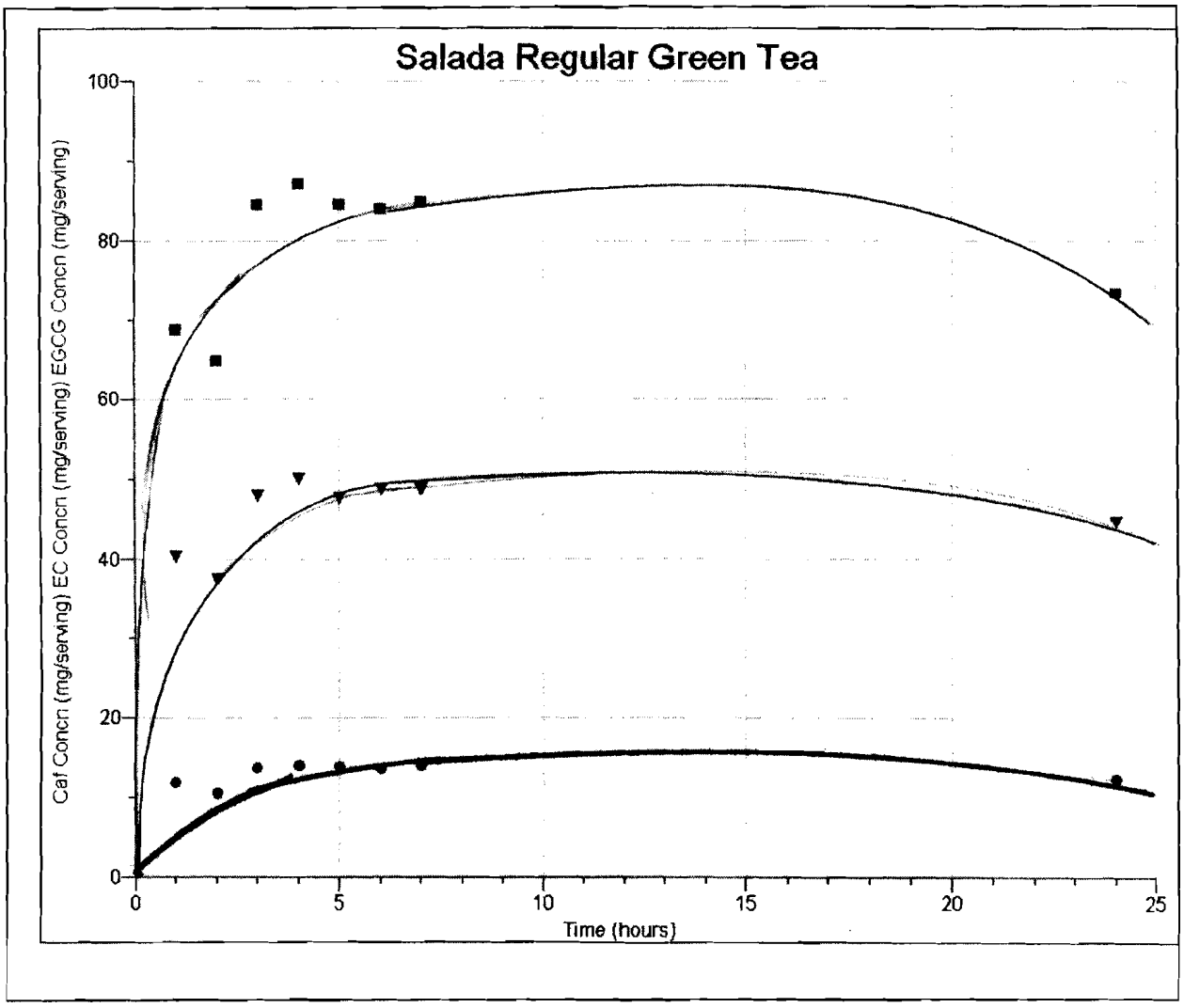


Figure 19. The concentration of caffeine, epicatechin (EC) and epigallocatechin gallate (EGCG) plotted against the time of extraction of Salada® caffeinated green tea at different extraction temperatures.

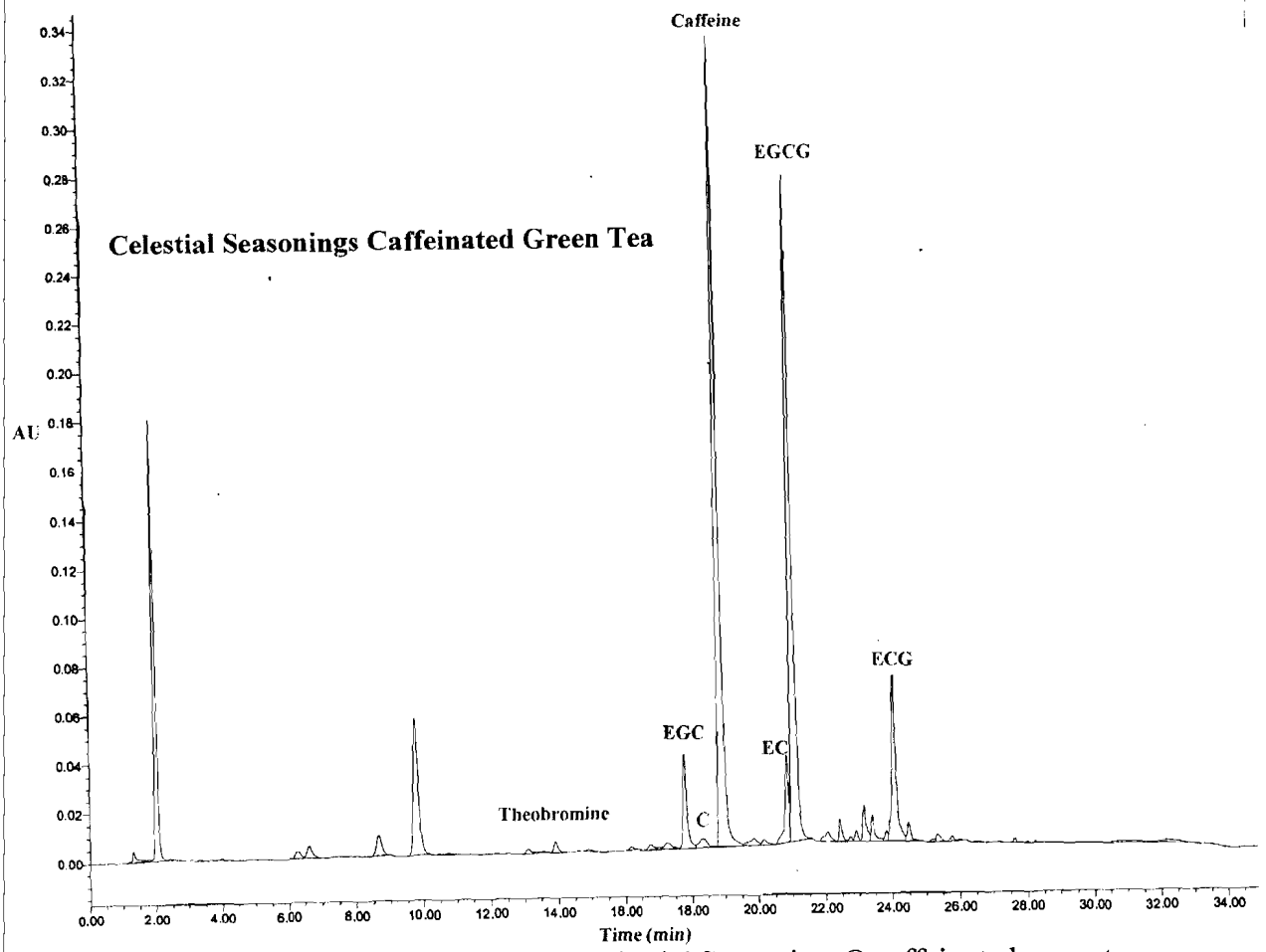


Figure 20. Chromatograph of Celestial Seasonings® caffeinated green tea

Salada® Green Tea Analysis

Salada® caffeinated green tea. The amounts of polyphenols and methylxanthines present in Salada® caffeinated and decaffeinated forms were analyzed and the results are shown in Tables 15 and 16, respectively. All the analyses were conducted per serving size (250 mL). Theophylline (Tp) was not detectable in any sample. Theobromine (Tb) concentrations remained almost constant with the increasing time at each constant temperature. The largest amounts of total polyphenols were found at 80 °C extraction temperature compared to other temperatures. For example, the highest extraction of total polyphenols, 239 mg/serving was found at extraction time of eight minutes at 80 °C. The caffeine concentrations increased up to 8 minutes extraction and then generally decreased at all temperatures. The concentrations of caffeine decreased as the temperature decreased from 100 °C to 90 °C at corresponding extraction times, and then increased at increasing corresponding times. For example, at a temperature of 100 °C for 2 minutes extraction the concentration of caffeine was 54.4 mg/serving and at 90 °C for 2 minutes were 42.3 mg/serving and at 85 °C for 2 minutes were 52.8 mg/serving.

The total polyphenol content generally increased in concentration from 2 minutes to six minutes at each corresponding temperature, and then declined at 10 minutes. The total polyphenol content increased from 187 mg/serving to 225 mg/serving as the time was increased from 2 minutes to 10 minutes at constant temperature of 100 °C. The largest amount of total polyphenols was attained at a temperature of 80 °C and 8 minutes (239 mg/ serving). No specific trend for EGCG content was observed. The amount of caffeine ranged from 41-59 mg/serving, EGCG was from 83-131 mg/serving, and the total polyphenol content was from 147-239 mg/serving. A high extraction efficiency was noted at temperature of 80 °C at 8 minutes both for the EGCG (131 mg/serving) and total polyphenol content (239 mg/serving), and caffeine was

found at a larger level (59.5 mg/serving) at a temperature of 100 °C. A study by Pera-Uzanalic and others (2006) showed that higher extraction efficiency occurred at 80 °C compared to 95 °C. Degradation of catechins is greater at higher temperatures (95 °C compared to 80 °C). Therefore, prolonged extraction time (more than 20 minutes) showed a decrease in the amount of catechins at 80 °C and 95 °C (Perva-Uzanalic et.al., 2006). For example, the concentration total polyphenols at 95 °C is 174 mg/serving compared to 192 mg/serving at 80 °C at same extraction time of 2 minutes.

Salada® decaffeinated green tea. The decaffeination process can be completed using CO_2 and water, a method known as effervescence. The amount of caffeine retained after decaffeination is said to be less than 5%. The amount of caffeine left after decaffeination ranges usually from 3-6 mg/serving, which is about 5% of the caffeine amount present in caffeinated green tea and this was observed with the current study as well, with levels ranging from 3.3 to 5.7 mg/250 mL serving. Theobromine and caffeine concentrations varied minimally with changes in time and temperatures. A greater concentration of total polyphenol content was extracted at 100 °C for 10 minutes (147 mg/serving) and the lowest concentration was at 85 °C for 6 minutes (75.5 mg/serving). For example, at a temperature of 100 °C, the concentrations of total polyphenols were larger compared to the other temperatures analyzed particularly at 10 minutes (147 mg/serving) (Table 16). The range of polyphenol content was 75 to 147 mg/serving size. The amount of decaffeination using supercritical carbon dioxide has been noted to remove 37.8 % of EGCG (Seok Park et al., 2007). Comparing the EGCG from caffeinated green tea to the decaffeinated green tea, the percentage of EGCG removed in the current study ranges from 38-49%, which is slightly higher than reported literature values. The typical decaffeination process also affects the amount of EGCG removed. At constant temperatures, the concentration

of catechins increased with an increase in time, whereas at a constant time, the concentration decreased with a decrease in temperature until 90 °C and then increased. For example, at a constant time of 6 minutes the concentration of total polyphenols at 100 °C, 95 °C, 90 °C, 85 °C, and 80 °C are 135 mg/serving, 117 mg/serving, 107 mg/serving, 75.5 mg/serving, and 86.2 mg/serving, respectively. Also for example, at a constant temperature of 90 °C, the concentration of total polyphenols at 2 minutes, 4 minutes, 6 minutes, 8 minutes and 10 minutes are 86.5 mg/serving, 93 mg/serving, 86.2 mg/serving, 94 mg/serving and 95.1 mg/serving, respectively.

Table 15

The Average Concentrations of all the Analytes in Salada® Caffeinated Green Tea in mg/250 mL Serving

Concentration (mg/serving)±SD	Tp[1]	Tb	Caf	C	EC	EGC	ECG	EGCG	Total polyphenols
At temp of 100°C									
2 minutes	n/a	1.0±0.1	54.4±2.0	45.5±2.1	20.1±0.6	7.1±0.3	23.1±1.6	91.4±2.0	187±6.5
4 minutes	n/a	1.0±0.1	57.3±2.0	48.7±1.9	21.6±0.6	8.3±0.4	25.3±1.7	94.4±2.0	198±6.5
6 minutes	n/a	1.3±0.1	59.5±2.0	54.7±2.2	22.4±0.4	9.2±0.3	29.2±1.3	96.4±1.9	212±6.2
8 minutes	n/a	1.4±0.1	55.3±2.1	50.3±2.2	23.3±0.5	11.4±0.4	30.9±4.8	100±2.2	216±3.4
10 minutes	n/a	1.3±0.1	57.5±1.7	50.5±2.2	21.3±1.0	12.5±0.4	37.0±1.4	104±2.3	225±7.2
At temp of 95 °C									
2 minutes	n/a	1.7±0.1	50.5±1.7	36.7±2.1	10.8±0.5	2.3±0.4	23.2±1.7	101±2.6	174±7.2
4 minutes	n/a	1.8±0.1	53.6±2.0	39.5±2.0	11.8±0.6	2.6±0.2	25.2±1.5	110±2.3	189±6.5
6 minutes	n/a	1.8±0.1	58.4±1.8	44.7±2.1	15.3±0.5	2.7±0.4	30.1±1.9	132±2.1	225±7.1
8 minutes	n/a	1.8±0.1	54.3±2.0	40.7±1.9	13.0±0.4	2.7±0.4	28.2±1.7	119±2.1	203±6.4
10 minutes	n/a	1.9±0.1	55.5±2.0	40.9±2.0	12.8±0.5	2.7±0.3	28.3±1.4	118±2.1	202±6.4
At temp of 90 °C									
2 minutes	n/a	1.5±0.1	42.3±2.0	33.5±2.2	8.8±5.8	12.4±0.6	19.9±1.7	83±2.2	157±8.9
4 minutes	n/a	1.7±0.1	49.7±1.9	37.6±2.1	14.4±0.6	13.4±0.6	26.0±1.6	103±2.0	194±6.5
6 minutes	n/a	1.9±0.0	50.4±2.0	36.7±2.1	13.4±0.5	12.2±1.1	22.1±1.6	99±2.0	183±6.5
8 minutes	n/a	1.7±0.1	49.4±2.0	36.5±1.9	14.3±0.5	12.6±0.7	23.3±1.7	102±1.8	189±6.9
10 minutes	n/a	1.7±0.1	47.7±1.9	36.8±2.0	13.4±0.5	15.1±0.7	24.0±2.1	97±2.0	187±6.2
At temp of 85 °C									
2 minutes	n/a	1.7±0.0	52.8±2.0	39.6±1.9	12.3±0.4	3.0±0.6	23.2±1.5	108±2.0	186±6.2
4 minutes	n/a	1.7±0.1	52.5±2.0	39.6±1.9	12.5±0.5	3.4±0.3	23.4±1.7	108±1.9	187±5.7
6 minutes	n/a	1.4±0.1	41.4±2.2	31.5±2.1	10.2±0.4	2.4±0.6	18.2±1.6	85±2.0	147±6.4
8 minutes	n/a	3.9±0.0	55.5±2.0	43.4±2.3	15.7±0.4	3.5±0.6	25.2±1.4	115±1.9	203±6.3
10 minutes	n/a	1.8±0.1	55.5±2.0	41.9±1.3	15.3±0.6	3.5±0.7	25.2±1.4	116±2.0	202±5.6
At temp of 80 °C									
2 minutes	n/a	2.0±0.1	51.5±2.0	41.6±2.4	14.3±0.7	7.7±0.6	27.1±1.4	101±2.0	192±6.9
4 minutes	n/a	2.3±0.1	57.5±2.0	47.4±1.9	16.8±0.6	8.9±0.8	30.9±1.7	119±2.3	224±6.9
6 minutes	n/a	2.2±0.1	54.5±2.0	44.7±2.2	14.7±0.6	8.7±0.6	30.1±1.9	116±2.0	215±7.0
8 minutes	n/a	2.3±0.1	58.3±1.9	48.4±1.8	17.4±0.5	9.3±0.9	34.2±1.6	131±2.1	239±6.3
10 minutes	n/a	2.2±0.1	56.4±2.0	47.4±1.6	16.4±0.6	9.5±0.8	32.2±1.6	124±2.0	229±6.0

[1]n/a=not available

Table 16

The Average Concentrations of all the Analytes in Salada® Decaffeinated Green Tea in mg/250 mL Serving

Concentration (mg/serving)±SD	Tp[1]	Tb	Caf	C	EC	EGC	ECG	EGCG	Tolal Polyphenols
At temp of 100 °C									
2 minutes	n/a	1.1±0.1	4.8±0.2	23.0±1.3	8.3±0.3	3.5±0.2	16.8±1.3	66.6±2.1	118±4.8
4 minutes	n/a	1.2±0.1	5.4±0.2	25.0±1.6	9.2±0.2	4.3±0.1	21.1±1.1	78.4±2.1	138±5.1
6 minutes	n/a	1.1±0.1	5.1±0.1	24.1±1.7	9.4±0.3	5.3±0.2	19.2±1.5	76.5±2.1	135±5.7
8 minutes	n/a	1.2±0.1	5.3±0.2	24.0±1.0	9.8±0.1	4.8±0.1	17.1±1.3	78.5±2.0	134±4.6
10 minutes	n/a	1.3±0.1	5.4±0.2	27.3±1.9	10.3±0.3	5.6±0.1	18.9±0.9	84.4±1.9	147±5.2
At temp of 95 °C									
2 minutes	n/a	1.2±0.1	4.7±0.2	18.4±0.6	6.6±0.2	1.8±0.1	12.8±0.6	55.3±1.9	95.0±3.4
4 minutes	n/a	1.2±0.1	5.5±0.2	20.4±0.6	6.2±0.3	1.8±0.1	15.1±0.6	63.7±2.1	107±3.7
6 minutes	n/a	1.3±0.1	5.1±0.1	21.6±0.5	8.1±0.1	2.1±0.1	15.8±0.5	69.2±1.9	117±3.3
8 minutes	n/a	1.2±0.1	4.7±0.2	19.8±0.7	7.4±0.2	1.9±0.1	15.9±0.6	66.7±1.8	112±3.4
10 minutes	n/a	1.2±0.0	5.6±0.2	21.2±0.6	7.7±0.3	2.2±0.1	16.2±0.3	67.3±1.8	115±3.0
At temp of 90 °C									
2 minutes	n/a	1.1±0.1	4.7±0.2	17.7±0.4	6.0±0.2	1.7±0.1	11.4±0.5	49.7±1.7	86.4±3.0
4 minutes	n/a	1.2±0.1	4.7±0.2	20.8±0.3	7.3±0.2	2.1±0.2	14.6±0.5	63.5±2.0	108±3.3
6 minutes	n/a	1.2±0.1	4.7±0.2	19.7±0.6	7.1±0.2	1.8±0.2	15.2±0.7	63.6±2.0	107±3.6
8 minutes	n/a	1.2±0.1	4.7±0.2	20.3±0.6	7.4±0.2	1.8±0.2	14.9±0.9	63.5±1.7	108±3.6
10 minutes	n/a	1.3±0.1	5.7±0.2	22.0±0.8	10.1±0.4	2.2±0.1	17.2±0.5	69.5±2.3	121±4.0
At temp of 85 °C									
2 minutes	n/a	1.0±0.1	3.8±0.2	16.7±0.4	6.4±0.3	2.3±0.1	11.0±0.5	49.7±1.9	86.1±3.2
4 minutes	n/a	1.2±0.1	4.2±0.2	18.6±0.5	7.4±0.2	2.7±0.1	11.9±0.5	54.4±2.0	95.0±3.4
6 minutes	n/a	0.8±0.1	3.3±0.2	14.5±0.5	6.1±0.2	2.0±0.2	9.4±0.4	43.5±2.1	75.5±3.4
8 minutes	n/a	1.0±0.1	4.2±0.2	17.3±0.4	7.1±0.3	2.4±0.2	12.5±0.5	55.7±1.8	95.1±3.1
10 minutes	n/a	1.2±0.1	4.8±0.2	20.1±0.7	7.7±0.2	2.8±0.2	15.5±0.3	66.6±2.0	113±3.4
At temp of 80 °C									
2 minutes	n/a	0.9±0.0	3.9±0.2	17.2±0.5	6.2±0.2	2.3±0.1	10.4±0.5	50.4±2.3	86.5±3.6
4 minutes	n/a	1.0±0.1	4.2±0.2	19.4±0.6	7.5±0.2	2.7±0.2	11.2±0.5	52.3±1.9	93.1±3.4
6 minutes	n/a	0.8±0.0	4.3±0.2	15.4±0.7	6.2±0.2	2.1±0.1	11.0±0.5	51.6±2.2	86.2±3.6
8 minutes	n/a	0.8±0.1	4.2±0.2	18.4±0.6	7.5±0.2	2.5±0.1	12.3±0.6	53.3±1.9	94.0±3.5
10 minutes	n/a	1.1±0.1	4.4±0.2	21.2±0.5	8.2±0.2	2.9±0.2	11.4±0.5	51.5±2.4	95.1±3.7

[1]n/a=not available

Celestial Seasonings® Green Tea Analysis

Celestial Seasonings® caffeinated green tea. Table 17 shows that the theobromine concentration was greater overall at a temperature of 100 °C and lowest at a temperature of 80 °C compared to other temperatures. The caffeine content overall increased with an increase in time at each temperature up to 8 minutes, and then it decreased. With a change in temperatures, the caffeine concentrations tended to increase from 80 °C to 90 °C and then decreased when temperatures were higher than 90 °C. Catechins increased in concentrations up to 8 minutes at each temperature and then decreased slightly. Total polyphenol concentration (280 mg/serving) was greatest at a temperature of 100 °C with an extraction time of 10 minutes. Total polyphenol concentrations typically decreased with a decrease of temperature at corresponding times. For example, total polyphenol concentrations at 100 °C, 95 °C , 90 °C, 85 °C , and 80 °C decreased as follows, 168 mg/serving, 145 mg/serving, 140 mg/serving, 106 mg/serving and 98.5 mg/serving, respectively at a constant extraction time of 4 minutes. The range of total polyphenols was from 97 - 280 mg/serving, EGCG was 51-110 mg/serving, and caffeine was 28-42 mg/serving. Compared to the Salada® caffeinated green tea, the range of total polyphenols is wider with EGCG, and caffeine lower in range. The differences in the amounts might be due the type of green tea leaves used, processing conditions, type of processing (bi-directional rolling or uni-directional rolling). The amount of tea leaves in the tea bag also affects the change in the concentrations of the polyphenols and methylxanthines. Salada® has a larger mass of tea leaves compared to Celestial Seasonings® by around 0.2 g. The package directions say to steep the tea bag in boiling water for 3 minutes. Comparing the amount of polyphenols at 4 minutes and 10 minutes at boiling temperature, 66% higher polyphenols was obtained. The concentration of

polyphenols at 4 minutes at boiling temperature is 168 mg/serving and at 10 minutes are 280 mg/serving, which is about a 66% increase (Table 17).

Celestial Seasonings® decaffeinated green tea. Table 18 shows that the amount of caffeine increased with an increase in time at a constant temperature, but the caffeine content did not follow a specific pattern with respect to the change in temperature across constant times. The concentrations of total polyphenols (Figure 20) increased with an increase in time at a constant temperature, and tended to decline with a decrease in temperature at a constant time. For example, at a constant temperature of 100°C, the concentrations at 2 minutes, 4 minutes, 6minutes, 8 minutes and 10 minutes are 76.1 mg/serving, 87.6 mg/serving, 97.2 mg/serving, 93.1 mg/serving, and 86.4 mg/serving, respectively. The amount of caffeine ranged from 28.4 mg/serving to 42.4 mg/serving in caffeinated green tea and 3.2 mg/serving to 6.4 mg/serving in decaffeinated green tea. The amount of caffeine retained falls to between 11 to 15% of the caffeine obtained from caffeinated green tea. The concentration of EGCG tended to increase as the extraction time increased and the leveled off similar to the trend shown by total polyphenols. The range of the concentrations of EGCG was from 41-93 mg/serving size. Compared to the caffeinated green tea, the concentration of EGCG was reduced to 15-21% in decaffeinated Celestial Seasonings® green tea. The decaffeination process did not affect the amount of catechins sustainably, however; the efficiency of removing caffeine is found to be less. The amount of total polyphenols decreased with a decrease in temperature at a constant time, but tended to increase with an increase in time at constant temperatures until 8 minutes and then decreased. The range of total polyphenols is from 90 to 177 mg/serving size. The larger amounts were obtained at a temperature of 100°C and particularly at an extraction time of 8 minutes.

Table 17

The Average Concentrations of all the Analytes in Celestial Seasonings® Caffeinated Green Tea in mg/250 mL Serving

Concentration (mg/serving)±SD	Tp[1]	Tb	Caf	C	EC	EGC	ECG	EGCG	Total Polyphenols
At temp of 100 °C									
2 minutes	n/a	1.2±0.1	30.4±2.3	31.1±0.7	11.2±0.8	4.3±1.0	12.7±1.2	55.2±0.8	165±2.7
4 minutes	n/a	1.2±0.1	29.6±1.5	31.6±1.2	11.4±1.2	4.6±0.9	13.3±1.0	56.9±2.5	168±3.9
6 minutes	n/a	1.5±0.1	37.5±1.3	42.7±1.3	15.4±0.8	5.2±0.7	22.0±0.5	89.1±2.9	242±3.7
8 minutes	n/a	1.6±0.1	39.9±2.1	49.2±0.2	16.8±0.7	5.6±0.3	27.5±1.4	106±2.5	276±6.4
10 minutes	n/a	1.7±0.1	42.4±3.5	47.4±0.1	16.8±0.6	5.9±0.5	26.5±1.0	110±1.5	280±2.3
At temp of 95 °C									
2 minutes	n/a	0.5±0.1	31.1±0.3	28.2±1.0	11.0±0.2	2.9±0.3	10.9±0.5	51.7±0.8	104±2.8
4 minutes	n/a	0.5±0.1	37.0±0.5	37.9±1.2	12.2±0.6	2.6±0.6	16.7±0.5	75.9±0.4	145±3.4
6 minutes	n/a	0.6±0.1	42.0±0.5	42.7±0.5	14.7±0.6	2.0±0.2	18.8±0.5	86.2±0.5	165±2.2
8 minutes	n/a	0.5±0.0	34.7±0.6	38.6±0.4	12.2±0.4	2.4±0.6	17.0±0.7	79.8±2.8	150±2.7
10 minutes	n/a	0.5±0.0	35.2±0.5	38.9±0.3	12.9±0.8	2.3±0.6	17.9±0.6	81.5±2.4	154±3.0
At temp of 90 °C									
2 minutes	n/a	0.5±0.1	36.0±0.5	31.4±1.4	11.1±0.6	1.7±0.6	13.3±0.6	62.0±2.4	120±3.5
4 minutes	n/a	0.6±0.1	38.0±0.3	39.2±0.9	12.8±0.8	1.4±0.6	15.1±0.3	71.9±2.1	140±3.3
6 minutes	n/a	0.6±0.1	41.3±1.1	43.4±0.7	14.0±0.1	1.7±1.1	17.8±0.6	83.2±5.5	160±3.0
8 minutes	n/a	0.6±0.1	38.1±0.3	37.8±1.0	12.8±0.7	1.7±0.7	15.7±0.5	73.8±2.1	142±3.7
10 minutes	n/a	0.6±0.1	38.1±0.9	39.7±0.4	12.9±0.8	3.2±0.7	18.1±0.6	81.6±5.5	156±3.1
At temp of 85 °C									
2 minutes	n/a	0.5±0.1	33.1±0.3	28.6±0.5	10.1±0.4	1.2±0.6	11.1±0.3	55.1±4.6	106±2.6
4 minutes	n/a	0.5±0.1	30.2±0.6	27.9±0.9	9.6±0.6	1.8±0.6	11.8±0.7	55.1±1.6	106±3.1
6 minutes	n/a	0.5±0.0	34.0±0.3	32.7±0.4	11.7±0.8	2.0±0.6	14.1±0.5	67.3±2.4	128±3.0
8 minutes	n/a	0.5±0.1	35.2±0.9	35.6±0.6	12.0±0.7	2.1±0.6	14.7±0.5	69.7±2.1	134±2.8
10 minutes	n/a	0.4±0.1	30.0±0.4	28.8±0.7	10.1±0.5	1.9±0.7	12.8±0.9	59.5±3.8	113±3.6
At temp of 80 °C									
2 minutes	n/a	0.4±0.0	32.0±0.6	26.5±0.8	9.1±0.5	0.6±0.1	9.2±0.5	52.3±2.0	97.9±1.9
4 minutes	n/a	0.4±0.0	29.2±0.7	25.2±0.3	9.5±0.5	1.3±0.1	10.3±0.7	52.2±3.3	98.5±2.0
6 minutes	n/a	0.4±0.1	32.5±0.6	30.5±1.4	11.0±0.5	1.5±0.1	12.3±0.4	64.0±3.0	119±2.9
8 minutes	n/a	0.4±0.0	33.5±0.6	33.2±0.5	11.5±0.5	1.6±0.1	13.3±0.5	64.2±2.9	124±1.7
10 minutes	n/a	0.3±0.0	28.4±1.0	26.0±0.6	9.3±0.6	1,3±0.0	12.5±0.6	56.9±4.4	106±2.5

[1]n/a=not available

Table 18

The Average Concentrations of all the Analytes in Celestial Seasonings® Decaffeinated Green Tea in mg/250 mL Serving

Concentration (mg/serving)±SD	Tp[1]	Tb	Caf	C	EC	EGC	ECG	EGCG	Total Polyphenol
At temp of 100 °C									
2 minutes	n/a	0.8±0.1	3.9±0.6	1.8±0.7	6.7±1.1	36.3±0.2	16.3±1.0	62.2±3.0	123±5.0
4 minutes	n/a	0.8±0.2	4.2±0.5	2.1±0.5	7.9±1.3	38.4±0.3	18.0±1.4	62.6±1.3	129±3.7
6 minutes	n/a	1.1±0.2	4.6±0.2	2.2±0.5	8.9±0.8	40.8±2.0	21.2±0.4	75.4±2.6	147±1.7
8 minutes	n/a	1.2±0.0	5.4±0.0	2.8±0.7	9.8±0.8	45.3±1.2	25.1±1.3	93.2±1.0	177±2.7
10 minutes	n/a	1.1±0.0	5.0±0.7	3.0±0.9	9.9±1.2	40.7±1.4	25.6±1.8	83.8±2.0	163±6.4
At temp of 95 °C									
2 minutes	n/a	0.8±0.1	3.2±0.2	1.5±0.4	7.5±1.5	28.7±0.7	16.5±1.4	51.7±1.3	106±1.6
4 minutes	n/a	1.0±0.2	3.3±0.5	1.9±0.3	7.2±0.4	35.9±0.9	16.1±2.6	62.9±3.3	124±6.2
6 minutes	n/a	0.9±0.1	3.7±0.8	1.8±0.7	7.1±0.9	36.7±0.4	17.9±1.0	68.4±4.4	132±5.7
8 minutes	n/a	1.3±0.0	3.5±0.9	2.1±0.7	7.9±1.2	39.4±0.3	20.3±0.3	73.3±3.3	143±5.1
10 minutes	n/a	0.9±0.0	3.5±0.6	2.0±0.4	7.8±1.1	35.0±0.7	19.1±0.1	68.3±1.5	132±1.4
At temp of 90 °C									
2 minutes	n/a	0.9±0.1	3.0±0.2	1.5±0.1	6.5±0.2	26.2±1.3	15.7±0.5	48.1±0.7	98±0.9
4 minutes	n/a	1.2±0.1	3.9±0.1	1.8±0.1	6.9±0.1	33.2±1.3	12.0±1.7	47.9±1.5	102±4.6
6 minutes	n/a	1.1±0.1	3.9±0.1	1.9±0.0	6.6±0.4	34.0±1.0	13.0±1.6	50.8±1.1	106±4.2
8 minutes	n/a	1.2±0.1	5.7±0.4	2.2±0.0	7.4±0.2	37.3±0.7	14.3±0.8	57.3±0.9	118±2.6
10 minutes	n/a	1.2±0.1	6.4±0.4	1.8±0.0	7.7±0.2	33.0±1.4	18.1±0.8	52.4±1.4	113±3.9
At temp of 85 °C									
2 minutes	n/a	1.1±0.0	3.8±0.4	1.9±0.0	6.1±0.2	29.1±0.3	11.6±0.7	41.3±0.4	90±0.7
4 minutes	n/a	1.2±0.0	4.2±0.7	2.2±0.0	6.8±0.0	30.6±1.3	13.3±0.1	44.5±2.6	97.4±2.7
6 minutes	n/a	1.2±0.0	4.5±0.5	2.0±0.1	7.2±1.5	31.0±2.3	13.9±0.3	45.4±0.9	99.5±4.5
8 minutes	n/a	1.3±0.0	4.7±0.5	2.9±0.1	9.9±1.9	33.9±0.8	15.2±0.5	51.8±1.6	114±2.5
10 minutes	n/a	1.4±0.0	4.9±0.6	3.0±0.1	10.6±0.9	38.9±0.6	17.3±0.7	59.7±1.0	130±1.3
At temp of 80 °C									
2 minutes	n/a	1.2±0.0	3.7±0.4	1.4±0.1	6.1±0.1	29.0±0.8	13.3±0.4	45.3±1.8	95.0±2.2
4 minutes	n/a	1.2±0.0	3.8±0.3	1.4±0.1	7.4±0.1	32.4±1.0	14.1±1.6	48.3±3.4	104±4.7
6 minutes	n/a	1.3±0.1	4.0±0.2	1.5±0.1	8.5±0.8	34.7±1.5	14.3±2.0	58.7±2.3	118±5.6
8 minutes	n/a	1.3±0.1	4.1±0.0	1.6±0.1	9.9±0.6	37.0±2.5	16.0±1.5	59.9±1.3	124±2.1
10 minutes	n/a	1.1±0.1	3.8±0.5	1.4±0.0	8.2±0.8	35.8±2.1	11.6±0.3	56.4±2.4	113±3.1

[1]n/a=not available

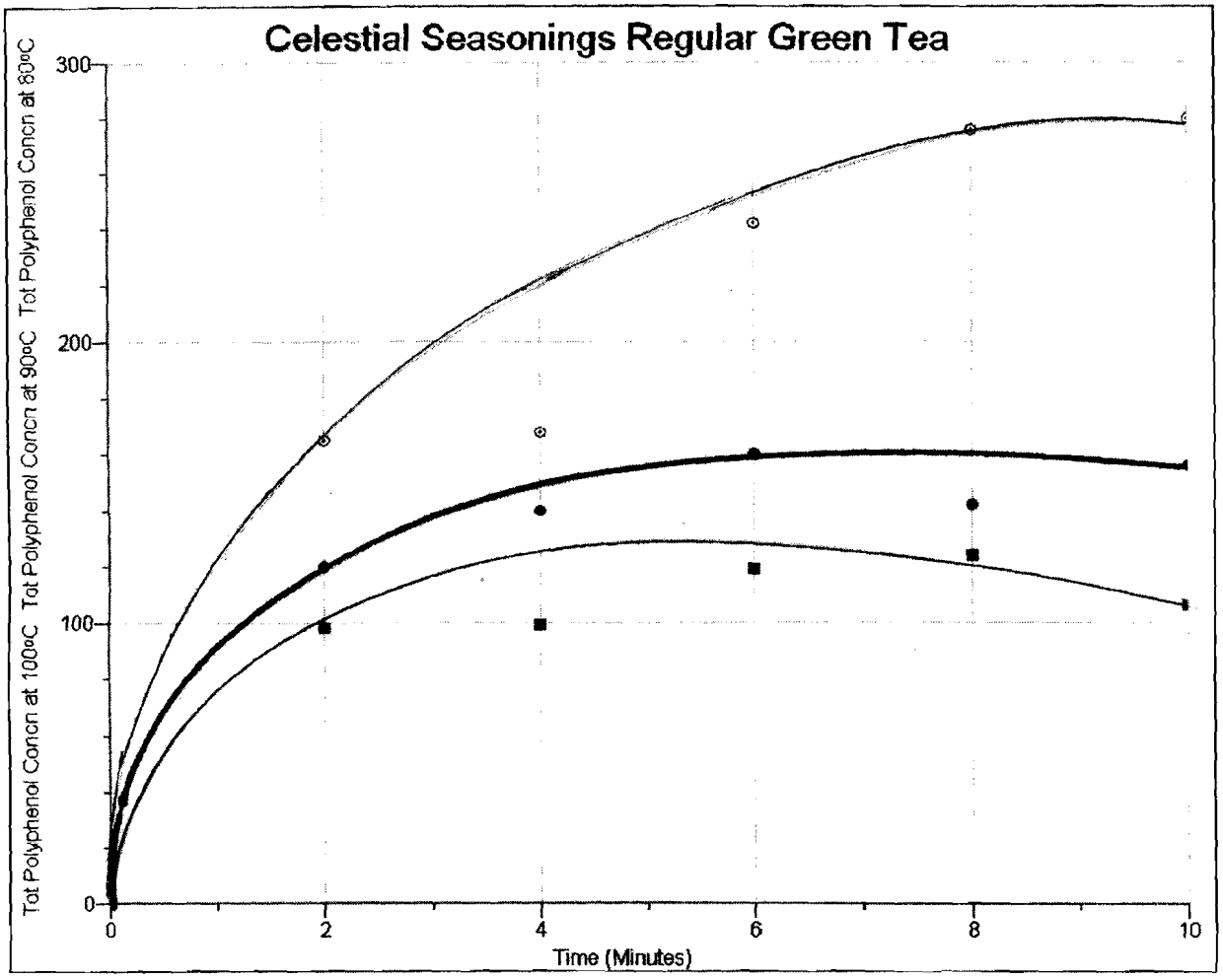


Figure 21. The concentration of total polyphenols plotted against the time of extraction of Celestial Seasonings® caffeinated green tea at extraction temperatures of 100 °C, 90 °C and 80 °C.

Bigelow® Caffeinated Green Tea

Bigelow® caffeinated green tea. Table 19 shows that the theobromine concentrations remained almost constant with level ranging from 0.9 to 1.7 mg/serving within each extraction condition. The extraction of caffeine, and total polyphenols increased with an increase in time until 8 minutes and then remained constant at constant temperatures; however, there was a decrease in concentrations with a decrease in temperature at constant times. The range of caffeine concentrations was from 20-31 mg/serving, EGCG was 23-47 mg/serving, and total polyphenol content was from 51-109 mg/serving. Comparing the caffeine content with the brands investigated, Bigelow® was found to have the lowest caffeine, EGCG and total polyphenol content compared to all other brands. Green tea is known for its health benefits due to its antioxidant properties and the benefits include: reducing the risk of cancer and coronary heart disease, which might be due the ability of catechins to prevent low density lipoprotein (LDL) from being oxidized; antibiotic effects; a possible role in treating humans with type 2 diabetes; preventive effects on chronic inflammatory diseases and neurological disorders (Chiu, 2006). When polyphenols are lower, any associated health benefits are also lower. Therefore, Bigelow® tea may not be as high in healthy antioxidants as the other brands studied. Larger amounts of caffeine, EGCG and total polyphenols were obtained at a temperature of 100 ^{o}C and up to an extraction time of 8 minutes. The company recommended brewing conditions of Bigelow® green tea brand is 85 ^{o}C for 3 to 5 minutes. When comparing the concentration at this extraction condition and the conditions at which the largest concentration was attained, the difference of only 2 mg was observed for total polyphenols and 7 mg for EGCG and 5 mg for

caffeine. This shows that the package directions might represent the brewing conditions at which maximum concentrations of polyphenols could be obtained.

Bigelow® decaffeinated green tea. The extraction of methylxanthines was almost constant with the change of time and temperatures and it was found that the polyphenol content increased with increasing time at constant temperature and decreased with a decrease in temperature. For example, the largest amount of total polyphenols (194 mg/serving) and EGCG (102 mg/serving) was obtained at an extraction temperature of 100°C at 10 minutes but decreasing the temperature to 80°C at 10 minutes only gave 155 mg/serving total polyphenol and 78.9 mg/serving EGCG. The caffeine left after the decaffeination process is 2.6 to 5.8 mg/serving size, which is about 15 to 18 percent of the caffeine found in the Bigelow® caffeinated green tea. Interestingly, the amount of EGCG was found to be greater in the decaffeinated green tea than in the caffeinated tea. The range of EGCG obtained was from 56 to 102 mg/serving and the total polyphenol content ranged from 112 to 194 mg/serving that is almost double the amount compared to the EGCG and total polyphenol content of the Bigelow® caffeinated green tea. The Salada® and Celestial Seasonings® caffeinated green tea showed greater amounts of polyphenols than the decaffeinated forms but Bigelow® showed the reverse effect which is very interesting. The decaffeinated green tea leaves mass was found to be almost the same as that of the caffeinated tea leaves of Bigelow® brand. The Bigelow® decaffeinated green tea (1.41 g/tea bag) had a lower mass of tea leaves compared to the Salada® (1.82 g/tea bag) and Celestial Seasonings® (1.64 g/tea bag) decaffeinated green teas. This implies that differences in the tea leaves mass might have had an affect on the change in the concentrations of polyphenols and methylxanthines.

Table 19

The Average Concentrations of all the Analytes in Bigelow® Caffeinated Green Tea in mg/ 250 mL Serving

Concentration[1] (mg/serving)	Tp	Tb	Caf	C	EC	EGC	ECG	EGCG	Total Polyphenol
At temp of 100 °C									
2 minutes	0.5±0.1	1.7±0.1	27.4±0.5	0.6±0.1	6.2±0.3	16.3±2.0	11.3±0.7	41.8±1.4	76.1±5.6
4 minutes	0.6±0.1	1.5±0.0	29.3±0.8	0.7±0.1	6.9±0.3	22.6±1.1	14.5±0.6	42.7±0.5	87.6±1.1
6 minutes	0.5±0.1	1.6±0.1	31.1±0.8	0.5±0.1	8.2±0.5	25.5±0.8	15.7±0.7	47.2±0.5	97.2±1.6
8 minutes	0.6±0.1	1.5±0.0	31.7±1.1	0.9±0.1	7.1±1.0	24.6±0.7	15.6±0.9	45.0±0.7	93.1±1.1
10 minutes	0.5±0.1	1.4±0.0	29.5±0.7	0.5±0.1	6.0±0.5	22.3±0.7	15.6±0.6	41.9±0.6	86.4±1.3
At temp of 95 °C									
2 minutes	0.5±0.1	1.3±0.1	26.8±0.8	0.8±0.1	6.0±0.7	17.7±1.1	10.3±0.3	24.9±1.1	59.6±0.9
4 minutes	0.4±0.1	1.4±0.0	27.9±1.0	0.6±0.1	5.0±0.5	15.2±1.0	10.1±0.9	29.6±1.0	60.5±1.6
6 minutes	0.7±0.1	1.4±0.1	30.1±0.7	0.9±0.1	9.0±0.1	27.3±1.0	13.1±0.7	33.6±1.0	83.9±0.8
8 minutes	0.7±0.1	1.5±0.1	31.1±0.7	1.1±0.2	17.2±0.5	30.7±1.4	15.2±1.0	44.5±0.5	109±1.4
10 minutes	0.7±0.1	1.5±0.1	29.3±0.8	0.8±0.1	13.4±0.7	24.7±3.2	12.1±0.7	39.8±0.8	90.9±4.6
At temp of 90 °C									
2 minutes	0.5±0.1	1.2±0.1	25.9±0.5	0.9±0.1	5.9±0.6	17.9±1.4	9.4±0.7	27.7±0.7	61.9±1.9
4 minutes	0.5±0.1	1.3±0.2	28.2±0.4	1.1±0.2	7.0±0.9	19.3±1.8	12.3±0.5	37.8±0.8	77.6±2.4
6 minutes	0.6±0.1	1.3±0.1	28.4±0.4	1.2±0.3	7.0±0.9	21.9±0.4	13.2±0.8	38.8±1.3	82.2±0.7
8 minutes	0.6±0.1	1.4±0.1	29.9±0.8	1.4±0.1	7.7±0.6	21.7±1.0	14.1±0.4	44.4±1.0	89.3±0.6
10 minutes	0.5±0.1	1.4±0.1	29.2±0.6	1.1±0.2	7.5±1.4	22.4±0.6	15.1±0.9	43.5±0.8	89.6±1.5
At temp of 85 °C									
2 minutes	0.3±0.1	1.2±0.3	21.6±1.7	1.2±1.0	5.3±1.6	17.6±0.4	12.4±4.8	25.6±2.6	62.1±5.5
4 minutes	0.5±0.1	1.5±0.1	25.9±1.8	2.2±0.4	5.8±0.6	24.1±0.5	17.6±6.2	35.6±1.5	85.3±7.1
6 minutes	0.4±0.1	1.4±0.2	26.4±0.5	1.6±0.4	5.6±0.3	22.6±0.2	19.5±6.5	36.5±1.7	85.8±4.6
8 minutes	0.4±0.1	1.3±0.2	27.5±0.3	2.4±0.2	6.3±0.8	24.3±1.8	19.1±5.6	38.9±2.7	90.9±6.8
10 minutes	0.4±0.1	1.3±0.0	28.6±0.7	2.8±0.9	6.6±0.9	25.3±1.3	20.7±6.6	39.2±3.8	94.6±4.9
At temp of 80 °C									
2 minutes	0.4±0.1	0.9±0.1	20.4±0.5	1.8±0.1	4.0±0.2	14.4±0.7	7.7±0.6	23.5±1.0	51.5±2.5
4 minutes	0.5±0.1	1.3±0.1	28.4±1.4	2.0±0.1	5.9±0.5	20.6±0.5	12.2±0.9	36.1±0.8	76.8±2.7
6 minutes	0.6±0.1	1.4±0.1	30.3±0.6	1.8±0.1	7.0±1.0	24.1±0.6	13.4±0.6	39.3±0.5	85.7±2.4
8 minutes	0.6±0.1	1.4±0.1	29.2±0.5	2.1±0.1	7.1±0.9	24.0±0.8	13.5±0.6	41.5±1.3	88.2±3.7
10 minutes	0.5±0.1	1.3±0.1	26.2±0.7	1.7±0.1	6.3±1.1	21.3±0.6	11.9±0.8	36.2±1.0	77.5±3.4

[1]average concentration ±SD

Table 20

The Average Concentrations of all the Analytes in Bigelow® Decaffeinated Green Tea in mg/250 mL Serving

Concentration[1] (mg/serving)	Tp[2]	Tb	Caf	C	EC	EGC	ECG	EGCG	Total Polyphenols
At temp of 100 °C									
2 minutes	n/a	1.6±0.1	4.6±0.2	3.8±0.2	9.9±0.8	30.5±1.0	23.4±1.9	76.6±1.2	144±3.1
4 minutes	n/a	1.8±0.2	5.4±0.3	4.2±0.3	10.6±0.9	34.3±2.0	27.7±1.8	87.8±1.5	165±3.8
6 minutes	n/a	1.3±0.2	5.8±0.3	4.4±0.4	11.6±0.5	36.7±2.1	30.5±2.1	96.6±0.8	180±4.8
8 minutes	n/a	1.5±0.3	5.3±0.6	4.7±0.3	10.3±0.8	34.4±2.3	30.5±2.0	97.0±1.6	177±1.8
10 minutes	n/a	2.0±0.2	5.7±0.5	5.0±0.3	12.0±0.8	42.3±2.2	32.6±2.0	102±0.8	194±3.6
At temp of 95 °C									
2 minutes	n/a	1.2±0.0	3.4±0.2	2.6±0.3	9.6±0.5	37.9±3.1	20.8±0.1	72.3±1.6	143±4.9
4 minutes	n/a	1.3±0.0	3.4±0.2	3.3±0.2	10.0±1.0	40.6±2.6	23.3±0.4	79.8±1.7	157±4.2
6 minutes	n/a	1.3±0.0	3.8±0.3	3.5±0.3	10.6±0.7	41.8±1.0	25.2±0.4	84.5±1.3	166±1.5
8 minutes	n/a	1.3±0.1	3.8±0.0	3.9±0.2	9.1±1.2	43.1±4.1	26.5±3.3	85.7±3.8	168±1.2
10 minutes	n/a	1.3±0.1	4.0±0.1	4.0±0.1	11.8±1.2	44.5±2.8	26.7±2.2	91.5±5.5	179±8.9
At temp of 90 °C									
2 minutes	n/a	1.4±0.2	2.6±0.3	2.1±0.3	8.4±1.5	29.8±3.3	16.5±2.5	61.2±2.8	118±9.4
4 minutes	n/a	1.5±0.2	2.9±0.1	2.4±0.3	9.8±1.6	33.3±3.4	21.3±1.1	64.7±7.4	131±1.2
6 minutes	n/a	1.7±0.0	3.2±0.0	3.2±0.4	10.5±1.1	37.6±0.4	24.4±1.4	80.7±1.4	156±2.8
8 minutes	n/a	1.5±0.4	3.1±0.3	2.7±1.2	11.3±0.6	37.0±1.4	24.4±0.9	82.0±2.5	158±6.5
10 minutes	n/a	1.6±0.2	3.2±0.4	2.4±0.3	9.9±1.6	33.3±3.4	22.5±1.9	76.9±7.5	145±1.4
At temp of 85 °C									
2 minutes	n/a	1.4±0.2	3.2±0.1	1.8±0.5	8.8±1.8	26.6±2.5	16.9±3.1	58.1±8.6	112±1.3
4 minutes	n/a	1.7±0.0	3.7±0.7	1.9±0.6	10.9±0.7	35.2±4.6	21.7±1.0	73.9±2.6	144±7.6
6 minutes	n/a	1.7±0.1	3.9±0.6	2.2±0.5	11.5±0.6	38.6±3.5	23.1±1.2	78.7±2.6	154±7.3
8 minutes	n/a	1.6±0.2	4.1±0.4	2.2±0.5	10.6±1.4	36.6±4.5	23.3±1.9	79.2±5.8	152±1.3
10 minutes	n/a	1.7±0.0	4.3±0.3	2.7±1.1	12.1±2.5	38.6±2.1	24.6±1.2	82.5±2.8	161±7.7
At temp of 80 °C									
2 minutes	n/a	1.5±0.2	3.3±0.3	1.5±0.6	8.6±0.7	31.3±0.2	16.0±1.3	56.2±4.0	114±5.9
4 minutes	n/a	1.5±0.3	3.6±0.5	1.7±0.4	9.0±1.6	33.8±1.9	17.6±2.1	66.7±6.3	129±5.8
6 minutes	n/a	1.8±0.1	3.9±0.6	1.9±0.6	11.3±0.6	36.6±3.6	21.2±2.1	76.5±3.4	148±8.9
8 minutes	n/a	1.7±0.1	4.0±0.4	1.9±0.8	10.1±0.4	41.5±4.5	22.6±0.5	78.0±2.3	154±3.1
10 minutes	n/a	1.7±0.2	4.3±0.4	2.2±1.3	11.9±2.6	38.8±3.5	23.3±1.8	78.9±6.4	155±1.5

[1]average concentration±SD
[2]n/a=not available

Comparing the caffeine content obtained in both the caffeinated and decaffeinated forms of Bigelow®, Celestial Seasonings® and Salada® green tea, it was noted that Salada® caffeinated had the largest amount of caffeine extracted at a temperature of 100°C for 10 minutes (58 mg/serving) followed by Celestial Seasonings® at 100°C for 10 minutes (42 mg/serving) and lowest in Bigelow® at 100°C for 10 minutes (30 mg/serving) (Figure 21). The amount of caffeine at the same extraction conditions was also compared and the results show that the amount of caffeine left was nearly 13%, 12% and 8% in Bigelow®, Celestial Seasonings® and Salada® green teas, respectively. Indicating that decaffeination process used in the Bigelow® brand was more efficient in removing the caffeine followed by Salada® and Celestial Seasonings® green teas.

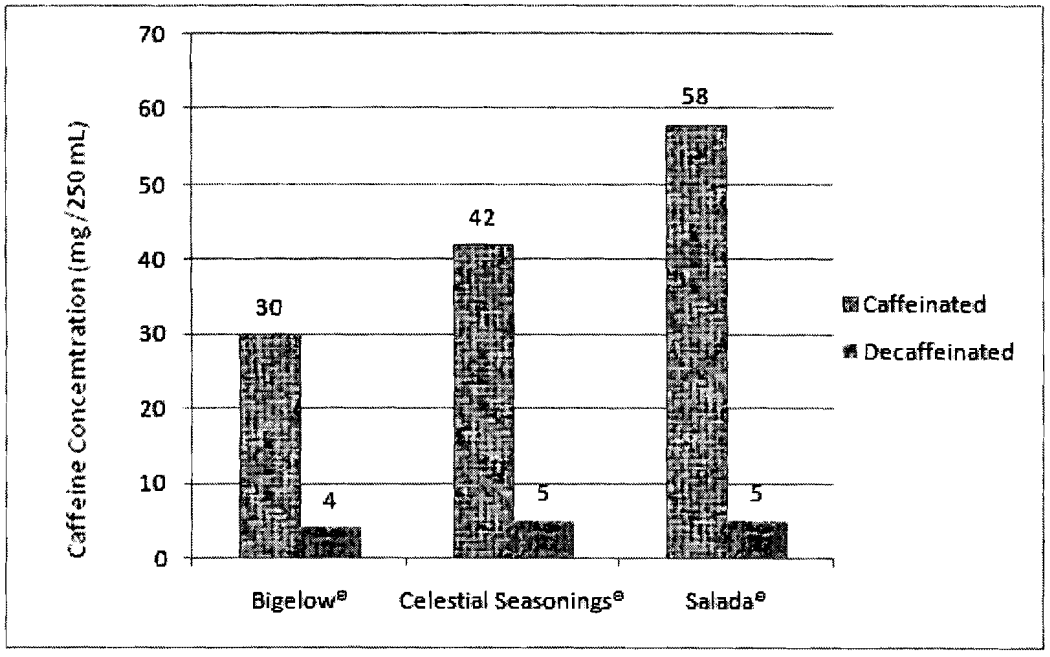


Figure 22. Mean caffeine concentrations (mg/serving) for both caffeinated and decaffeinated forms of Bigelow®, Celestial Seasonings® and Salada® green teas at extraction conditions of 10 minutes at 100°C.

EGCG is the most investigated polyphenol due to its associated health benefits including purported cancer preventive properties. A study conducted by Jung and Ellis (2001) showed that EGCG is shown to inhibit tumor invasion and angiogenesis in cells. Salada® was found to have the largest concentration (124 mg/serving at 80°C, 10 minutes) of EGCG compared to the other brands (Figure 22) of tea in the current study. Comparing EGCG among other brands, interestingly Bigelow® was found to have larger amounts of EGCG in the decaffeinated form (102 mg/serving) compared to the caffeinated green tea (47 mg/serving). This also added to the increase in the total polyphenol content (Figure 23) of Bigelow® decaffeinated green tea compared to the caffeinated tea.

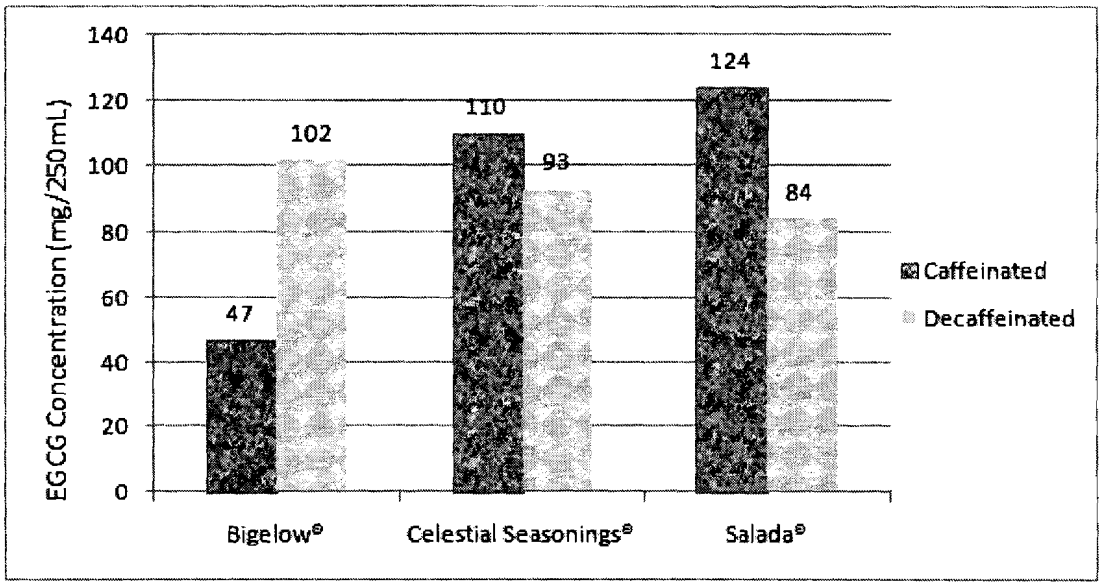


Figure 23. Mean EGCG concentrations (mg/serving) for both caffeinated and decaffeinated forms of Bigelow®, Celestial Seasonings® and Salada® green teas at highest extraction conditions.

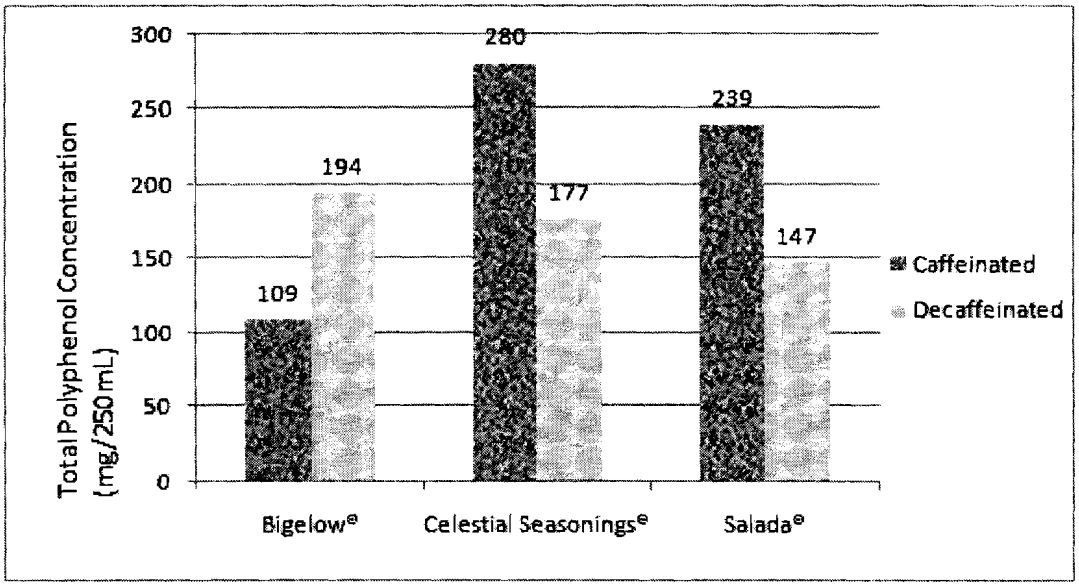


Figure 24. Mean total polyphenol concentrations (mg/serving) for both caffeinated and decaffeinated forms of Bigelow®, Celestial Seasonings® and Salada® green teas at highest extraction conditions.

Chapter V: Conclusion

The purpose of the study was to identify and quantify polyphenols and methylxanthines in three brands of green tea (Bigelow®, Salada® and Celestial Seasonings®) in both caffeinated and decaffeinated types at different extraction time and temperature conditions. The polyphenols and methylxanthines were analyzed by HPLC. All the concentrations were calculated in milligrams per 250 mL serving for analyzing amount of compounds per one cup of green tea for each bag. The analysis was carried out in triplicate for all the extraction conditions for each type of green tea analyzed. It is known that green tea has health benefits due to the antioxidant properties of the compounds in it.

Overall, polyphenols increased with increasing extraction temperature at constant time and decreased with decrease in temperature. This was observed for the all brands of green teas tested. Interestingly, Bigelow® decaffeinated showed larger amounts of EGCG and total polyphenols compared to the caffeinated form which was not seen in other two brands (Salada® and Celestial Seasonings®). All the brands used the same method of decaffeination for removal of caffeine. The mass of tea leaves per bag was found to be higher in caffeinated forms compared to the decaffeinated forms. Salada® caffeinated green tea has more concentrations of caffeine then followed by Celestial Seasonings® and then by Bigelow®. The higher amount of tea leaves in Salada® might be one of the reasons for it. Overall, the largest concentration of total polyphenols was observed in Celestial Seasonings® followed by Salada® and lowest in Bigelow® green tea.

This study was also aimed to identify and quantify the amount of polyphenols and methylxanthines at different extraction times of 1 hour through 24 hours at room temperature.

The concentrations of polyphenols tended to increase until 7 hours of extraction time and then decreased after 24 hours had elapsed. The concentrations of caffeine remained almost constant.

This study determined that larger concentrations of beneficial polyphenols are found in tea brewed for long periods of time (10 minutes) at higher temperatures (100°C). When steeping tea bags in room temperature water, it could be beneficial to steep until 7 hours and then refrigerate the tea. The decrease in concentrations after this time might be due to the oxidation of polyphenols after longer exposure to air. It is also very interesting that in Celestial Seasonings®, the concentrations of EGCG after 24 hours did not decrease while the decrease after 24 hours was observed in other brands. The presence of vitamin C which may protect EGCG from oxidation might be the reason for this. Research on the presence or absence of vitamin C and its role in protecting polyphenols from oxidation could help resolve this question.

If decaffeinated teas are to be chosen, then Bigelow® provides greater polyphenol concentrations. This study shows that consumers can enjoy health benefits of green tea even steeping for long periods of time at room temperature. Based on this research further steps are recommended to learn more about the polyphenols and their availability.

Recommendations

The following steps are recommended for further research

1. Conduct sensory evaluation of the tea brewed at different extraction conditions
2. Identify and quantify polyphenol content of green tea at each hour interval for 24 hours to observe at what time point the polyphenols tend to decrease.
3. Identify and quantify polyphenols / methylxanthines in food products that add green tea extracts.

4. Identify and quantify polyphenols when green tea leaves are heated in water to 100°C and then cooled over periods of time.
5. Determine the effect of different decaffeination processes on the polyphenols of green tea.
6. Determine the effect of extraction parameters on polyphenols of other teas like white tea/black tea/ oolong teas.

References

An, B., Kwak, J., Son, J., Park, J., Lee, J., Jo, C. et al. (2004). Biological and anti-microbial activity of irradiated green tea polyphenols. *Food Chemistry, 88,* 549-555.

Andao. (n.d). Retrieved January 15, 2008, from http://www.andaotea.com/andaopamphlet.pdf

Bonoli, M., Pelillo, M., Gallina Toschi, T., & Lecker, G. (2003). Analysis of green tea catechins: Comparative study between HPLC and HPCE. *Food Chemistry*, *81*, 631-638.

Borrelli, F., Capasso, R., Russo, A., & Ernst, E. (2004). Systematic review: Green tea and gastrointestinal cancer risk. *Amimnet Pharmacol Ther, 19,* 497-510.

Bushman, J. L. (1998). Green tea and cancer in humans: A review of the literature. *Nutrition and Cancer, 31*(3), 151-159.

Celestial Seasonings® caffeine free tea. (n.d). Retrieved January 18, 2008, from http://www.vitacost.com/celestialseasonings.html

Chen, H., Zhang, M., & Xie, B. (2005). Components and antioxidant activity of polysaccharide conjugate from green tea. *Food Chemistry, 90,* 17-21.

Chen, X., & Anderson, J. J. B. (2002). Isoflavones and bone: Animal and human evidence of efficacy. *J Musculoskel Neuron Interact, 2*(4), 352-359.

Chiu, S. (2006). Is green tea good for you? *Journal of Food Science Education*, *5*, 70-71.

Copeland, E. L., Clifford, M. N., & Williams, C. M. (1998). Preparation of (-)epigallocatechin gallate from commercial green tea by caffeine precipitation and solvent partition. *Food Chemistry*, *61*, 81-87.

Dreosti, I. E. (n.d.). Bioactive ingredients: Antioxidants and polyphenols in tea. *Nutrition Reviews, 54*(11), 51-58.

Ferruzzi, M. G., & Green, R. J. (2006). Analysis of catechins from milk-tea beverages by enzyme assisted extraction followed by high performance liquid chromatography. *Food Chemistry, 99,* 484-491.

Friedman, M., Kim, S. Y., Lee, S. J., Han, G. P., Han, J. S., Lee, K. R et al. (2005). Distribution of catechins, theaflavins, caffeine, and theobromine in 77 teas consumed in the United States. *Journal of Food Science, 70*(9), 550-559.

Friedman, M., Levin, C. L., Choi, S. H., Kozukue, E., & Kozukue, N. (2006). HPLC analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: Comparison of water and 80% ethanol/water extracts. *Journal of Food Science, 71*(6), 328-337.

Fujiki, H., Suganuma, M., Okabe, S., Sueoka, N., Komori, A., Sueoka, E. et al. (1997). Cancer inhibition by green tea. *Mutation Research, 402,* 307-310.

Fujiki, H., Suganuma, M., Matsuyama, S., & Miyazaki, K. (2005). Cancer prevention with green tea polyphenols for the general population and for patients following cancer treatment. *Current Cancer Therapy Reviews, 1,* 109-114.

Ito, R., Yamamoto, A., Kodama, S., Kato, K., Yoshimura, Y., & Matsunga, A. (2003). A study on the change of enantiomeric purity of catechins in green tea infusion. *Food Chemistry, 83,* 563-568.

Jung, Y. D., & Ellis, L. M. (2001). Inhibitions of tumor invation and angiogenesis by epigallocatechin-gallate (EGCG), a major component of green tea. *International Journal of Experimental Pathology, 82,* 309-316.

Kai, O. C., Chi, C. W., Michael, S. R., Kwong ,W. C., & Chi, P. P. (2004). Determination of catechins and catechin gallates in biological fluids by HPLC with coulometric array detection and solid phase extraction. *Analytical Chemical Acta, 510,* 69–76.

Kasper, K. (2006). *Identification and quantification of flavanols and methylxanthines in chocolates with different percentages of chocolate liquor*. Menomonie, WI: University of Wisconsin-Stout.

Lee, S., Park, M. K., Kim, K. H., & Kim, Y. S. (2007). Effect of supercritical carbon dioxide decaffeination on volatile components of green teas. *Journal of Food Science*, *72*(7), 497-502.

Li, H., Yashiki, S., Sonoda, J., Lou, H., Ghosh, S. K., Bymes, J. J. et al (2000). Green tea polyphenols induce apoptosis *in vitro* IN peripheral blood T lymphocytes of adult T-cell leukemia patients. *Jpn. J. Cancer Res., 91,* 34-40.

Li, J., & Jiang, Y. (2007). Litchi flavonoids: Isolation, identification and bilogical activity. *Molecules, 12,* 745-758.

Liang, H., Liang, Y., Dong, J., Lu, J., Xu, H., & Wang, H. (2007). Decaffeination of freash green tea leaf *(Camellia sinenis)* by hot water treatment. *Food Chemistry, 101,* 1451-1456.

Majchrzak, D., Mitter, S., & Elmadfa, I. (2004). The effect of ascorbic acid on total antioxidant activity of black and green teas. *Food Chemistry, 88,* 447-451.

Mandel, S., Weinreb, O., Amit, T., Youdim, M. B. H. (2004). Cell signaling pathways in the neuroprotective actions of green tea polyphenol (-) – epigallocatechin-3-gallate: implications for neurodegerative diseases. *Journal of Neurochemistry, 88,* 1555-1569.

Methylxanthines. (n.d). Retrieved January 15, 2008, from http://www.biology-online.org/dictionary/Methylxanthines

Murakami, M., Yamaguchi, T., & Matora, T. (2003). Effects of Ascorbic Acid and α-Tocopherol on Antioxidant Activity of Polyphenolic Compounds. *Journal of Food Science, 68*(5), 1622-1625.

Nakachi, K., Suemasu, K., Suga, K., Takeo, T., Imai, K., & Higashi, Y. (1998). Influence of drinking green tea on breast cancer malignancy among Japanese patients. *Jpn. J. Cancer Res., 89,* 254-261.

New cancer research. (n.d). Retrieved January 15, 2008, from http://www.newcancerresearch.com/Website_05022001.pdf

Okabe, S., Suganuma, M., Hayashi, M., Sueoka, E., Komori, A., & Fujiki, H. (1997). Mechanisms of growth inhibition of humans lung cancer cell line, PC-9, by tea polyphenols. *Jpn. J. Cancer Res, 88,* 639-643.

Okabe, S., Ochiai, Y., Aida, M., Park, K., Kim, S., Nomura, T. et al. (1999). Mechanistic aspects of green tea as a cancer preventive: Effect of components on human stomach cancer cell lines. *Jpn. J. Cancer Res, 90,* 733-739.

Pelillio, M., Biguzzi, B., Bendini, A., Gallina Toschi, T., Vanzizni, M., & Lecker, G. (2002). Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins. *Food Chemistry, 78*, 369-374.

Pelillio, M., Biguzzi, B., Bendini, A., Gallina Toschi, T., Vanzizni, M., & Lecker, G. (2004). An investigation in the use of HPLC with UV and MS-electrospray detection for the quantification of tea catechins. *Food Chemistry, 87,* 465- 470.

Perva-Uzunalic, A., Skerget, M., Knez, Z., Weinreich, B., Otto, F., & Gruner, S. (2006). Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. *Food Chemistry, 96,* 597-605.

Record, I. R., & Lane, J. M. (2001). Simulated intestinal digestion of green and black teas. *Food Chemistry, 73,* 481-486.

Reznichenko, L., Amit, T., Youdim, M. B. H., & Mandel, S. (2005). Green tea polyphenol (-)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. *Journal of Neurochemistry, 93,* 1157-1167.

Seok Park, H., Jin Lee, H., Hye Shin, M., Won, K., Lee, H., Kim, Y. et al. (2007). Effects of cosolvents on the decaffeination of green tea by supercritical carbon dioxide. *Food Chemistry, 105,* 1011-1017.

Sharma, V., Gulati, A., & Ravindranath, S. D. (2006). Extractability of tea catechins as a function of maufacture procedure and temperature of infusion. *Food Chemistry, 93,* 141-148.

Silvia, M., Orly, W., Tamar, A., & Moussa B. H. Y. (2004). Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. *Journal of Neurochemistry, 88,* 1555-1569.

Suminori, K. (1992). Green tea and colon cancer. *Jpn. J. Cancer Res, 83,* 669.

Tea processing. (n.d). Retrieved September 29, 2007, from http://www.enjoyingtea.com/teaprocessing1.html

Theivendran, S., Hettiarachchy, N. S., & Johnson, M. G. (2006). Inhibition of listeria monocytogenes by nisin combined with grape seed extract or green tea extract in soy protein film coated on turkey frankfurters. *Journal of Food Science, 71*(2), 39-44.

Wang, H., & Helliwell, K. (2000). Epimerization of catechins in green tea infusions. *Food Chemistry, 70,* 337-344.

Wang, H., Helliwell, K., & You, X. (2000). Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. *Food Chemistry, 68,* 115-121.

Weisburger, J. H. (1996). Tea antioxidants and health. In E. Andenas & L.Packer (Eds.), *Handbook of antioxidants* (pp. 469-471). New York, NY: Marcel Dekker, Inc.

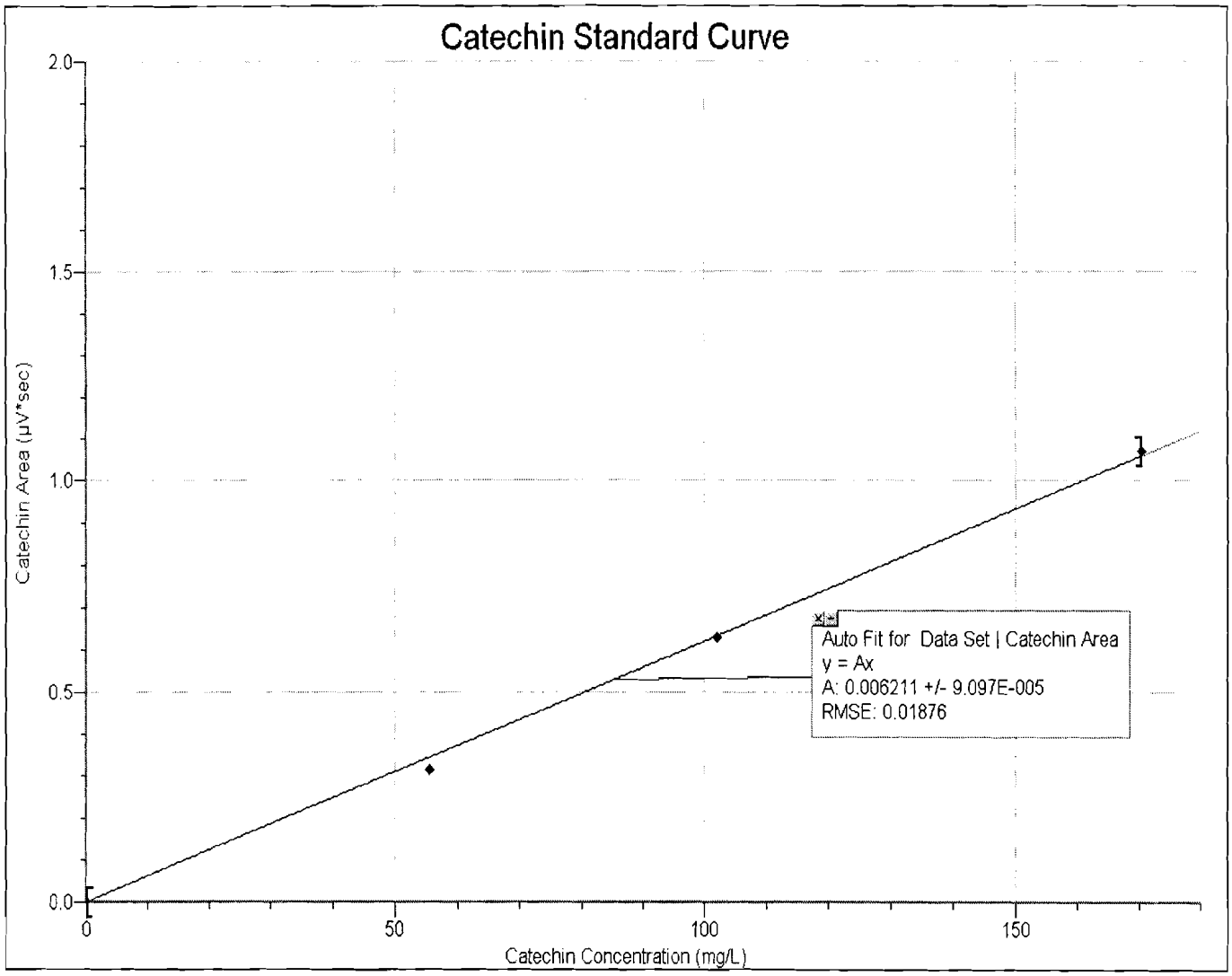
Xin, W., Shi, H., Zhao, B., & Hou, J. (1995). Studies on green tea polyphenol's antioxidative and protective effects on biomembranes. A.S.H. Ong, E. Niki, L. Packer (Eds), *Nutrition, Lipids, Health, and Disease* (pp. 67). Chapaign, IL: AOCS Press.

Yang, X. R., Ye, C. X., Xu, J. K., & Jiang, Y. M. (2007). Simultaneous analysis of purine alkaloids and catechins in *Camellia sinensis, Camellia ptilophylla* and *Camellis assamica* var. *kucha* by HPLC. *Food Chemistry, 100,* 1132-1136.

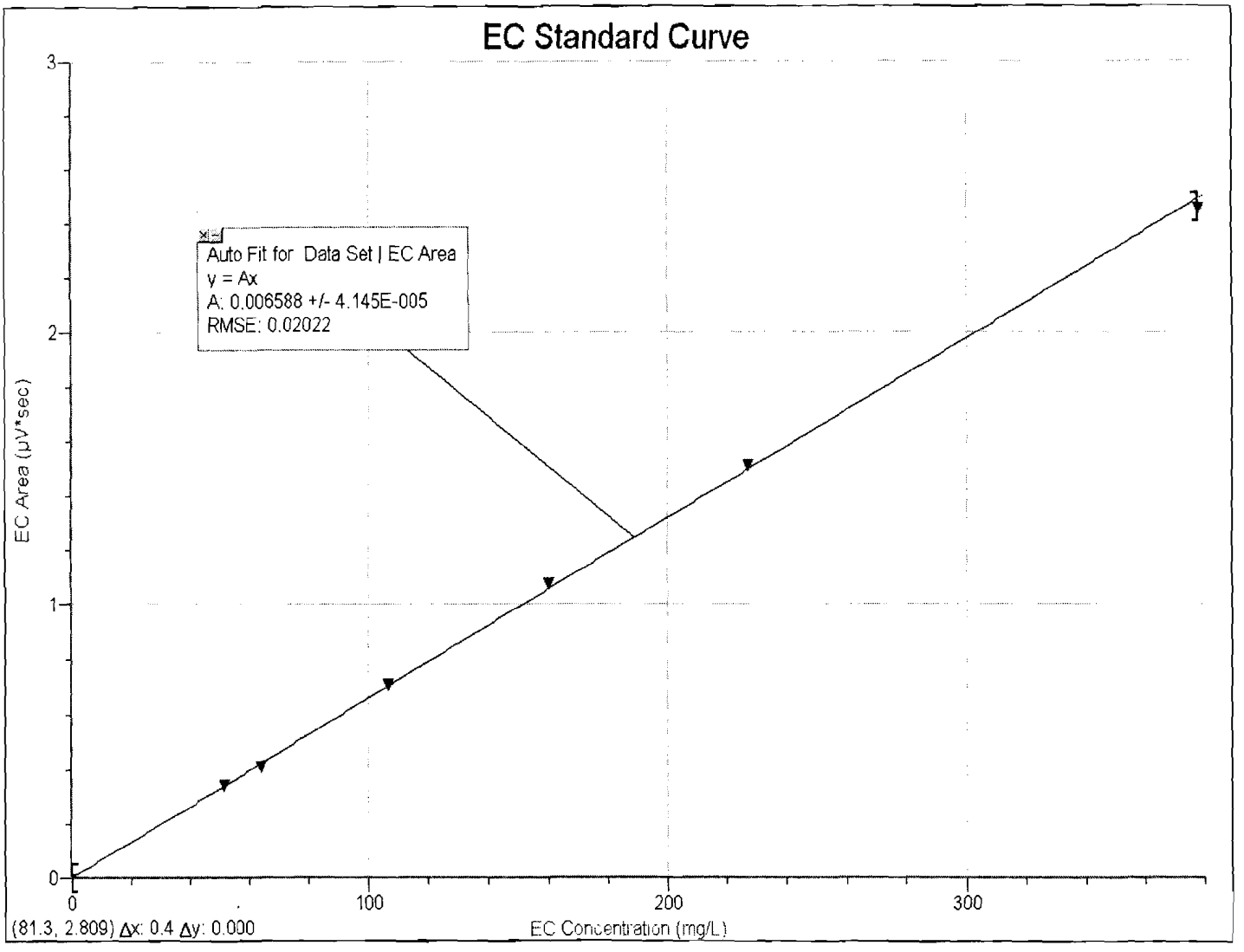
Yoshida, Y., Kiso, M., & Goto, T. (1999). Efficiency of the extraction of catechins from green tea. *Food Chemistry, 67,* 429-433.

Yusuf, N., Irby, C., Katiyar, S. K., & Elmets, C. A. (2007). Photoprotective effects of green tea polyphenols. *Photodermatol Photoimmunol Photomed, 23,* 48-56.

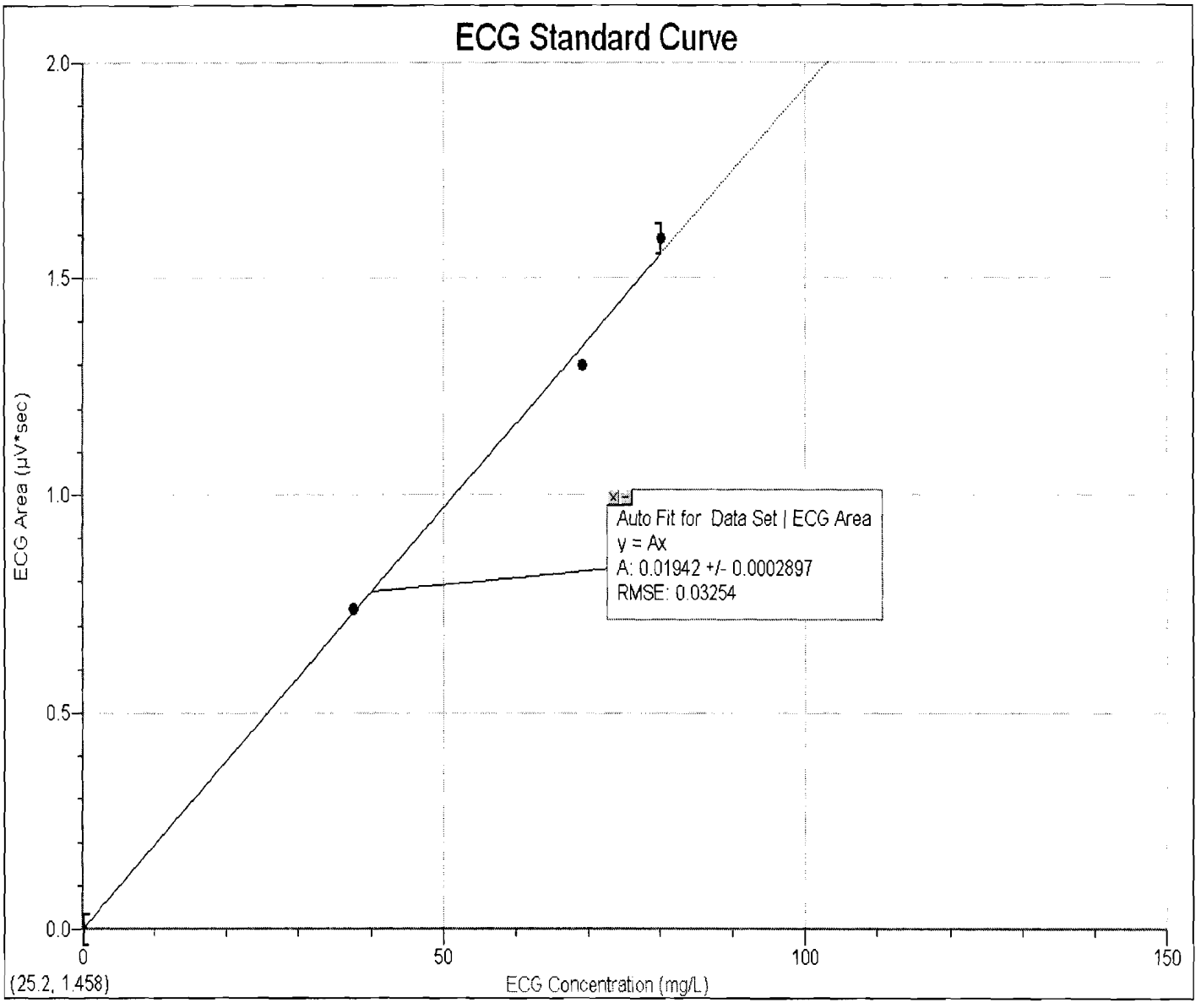
Appendix A: Catechin (C) Standard Curve



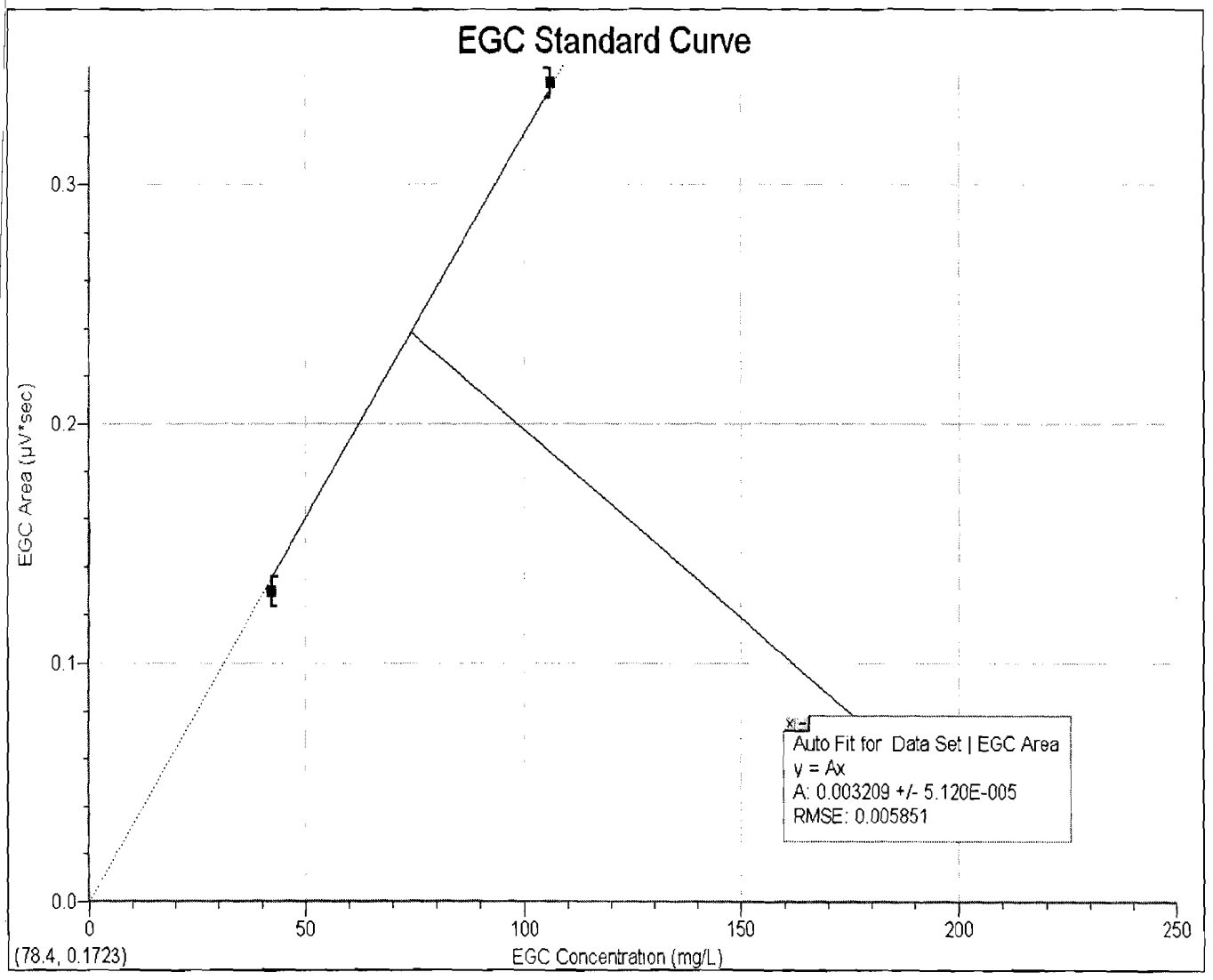
Appendix B: Epicatechin (EC) Standard Curve



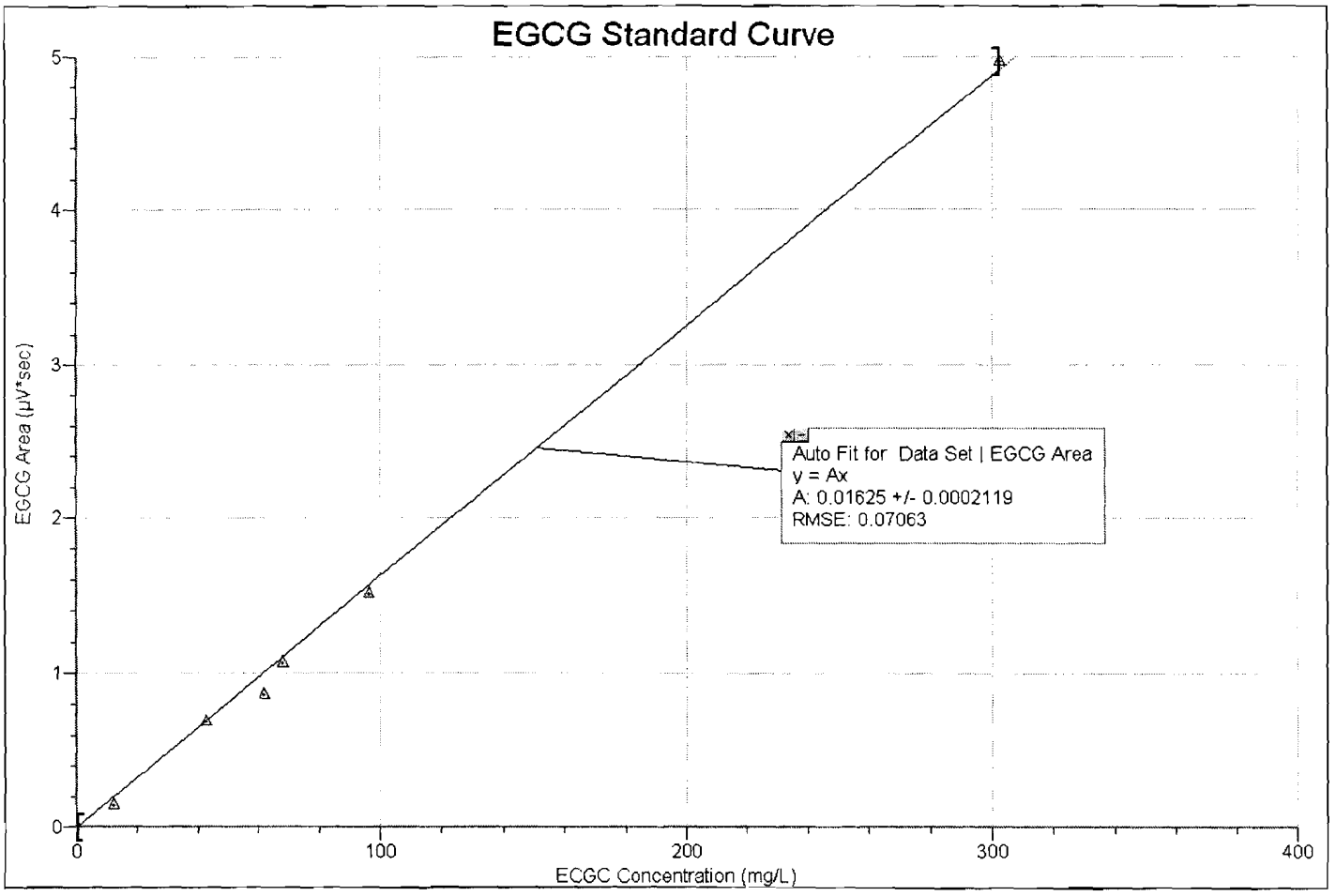
Appendix C: Epicatechin Gallate (ECG) Standard Curve



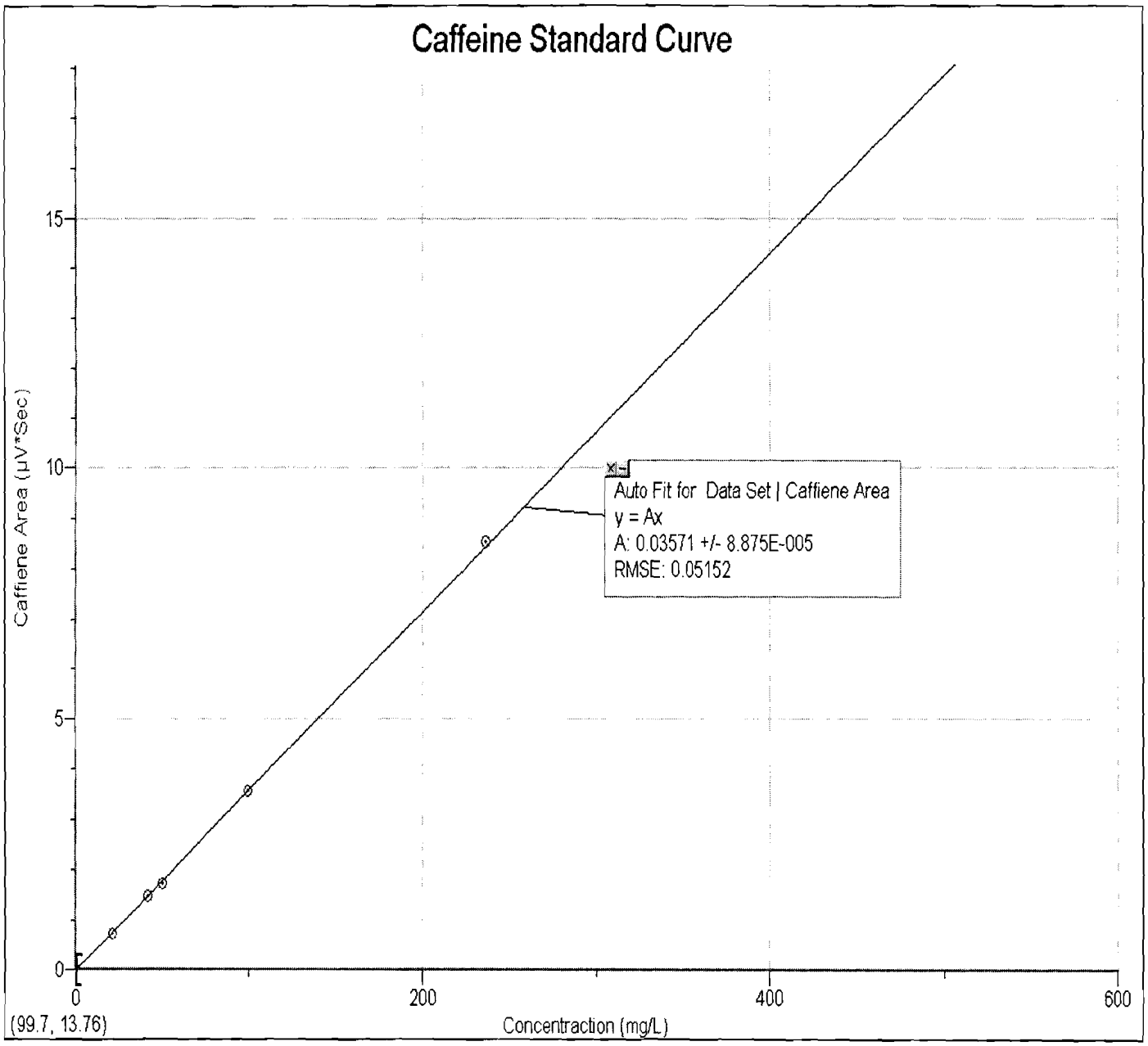
Appendix D: Epigallocatechin (EGC) Standard Curve



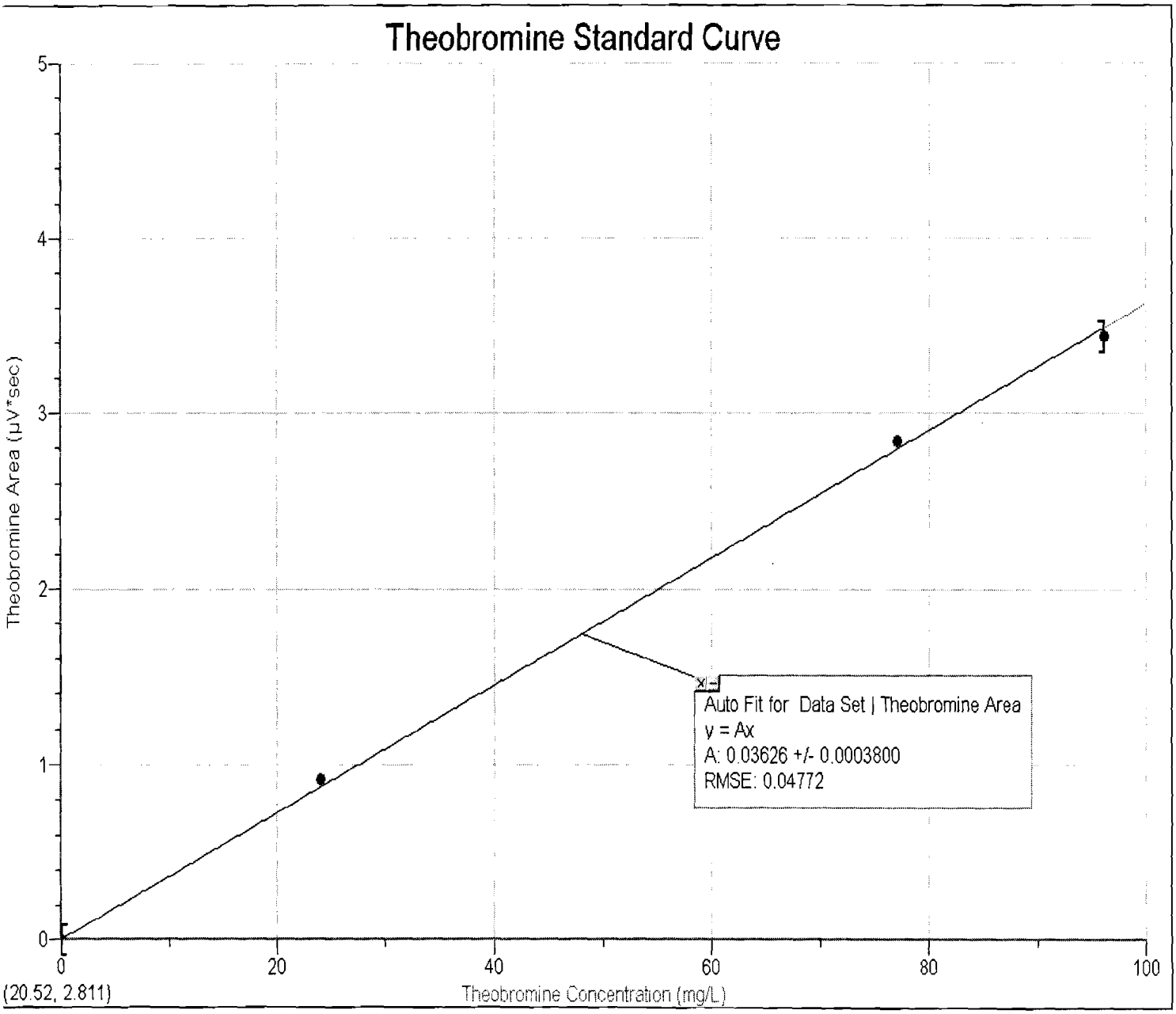
Appendix E: Epigallocatechin Gallate (EGCG) Standard Curve



Appendix F: Caffeine (Caf) Standard Curve



Appendix G: Theobromine (Tb) Standard Curve



Appendix H: Theophylline (Tp) Standard Curve