Polyphenol and Caffeine Concentrations Found in Lipton® White Tea with Blueberry and Pomegranate

by

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Abstract

The popularity of tea has stood the test of time. Today, tea is enjoyed around the world and its consumption reflects local preference and tradition. For the past three decades, epidemiologists have observed lower risks of cancer, cardiovascular disease, and osteoporosis in populations that drink tea frequently. The purpose of the study was to identify and quantify polyphenolic and methylxanthine concentrations in Lipton® White Tea flavored with Blueberry and Pomegranate at different steeping time and temperature conditions. The white tea was brewed with spring water in triplicate with varying steeping times (2-minutes, 5-minutes, 10-minutes, and 24-hours) and initial water temperatures (80°C, 85°C, 90°C, 95°C, and 100°C) and remained, to complete the extraction process, at room temperature. Reverse-phase high

performance liquid chromatography (HPLC) analysis was used to quantify tea polyphenol and methylxanthine components. Under research conditions, ideal steeping time and temperature for Lipton® white tea were found to be 24 hours at 100°C since this allowed the largest concentration of EGCG (30.4mg/250mL), catechin (22.0mg/250mL), and caffeine (167.3mg/250mL) to be expressed. White tea is a natural source of health promoting elements. This study‘s findings assist in providing steeping conditions having greatest concentration of health promoting polyphenols and methylxanthines in white tea.

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Chapter I: Introduction

Tea represents, after water, the most widely consumed beverage in the world (Stengl, 2007). Although this is true, many people still have misconceptions of what tea really is. The Merriam-Webster dictionary defines tea as a shrub called *Camellia sinensis,* of the tea family Theaceae, cultivated especially in China, Japan, and the East Indies from which the leaves, leaf buds, and internodes of the tea plant are prepared and cured for consumption. The tea is then classed according to its method of processing into one set of types (as white tea, green tea, black tea, or oolong tea). In Merriam-Webster‘s second definition, tea is an aromatic beverage prepared from tea leaves by infusion with boiling water. With these two definitions, it is clear that tea at its truest state is made with from *Camellia sinensis* and manufactured with different methods to produce varied products.

Reported from the China-Japan, Hindustani Centers of Diversity, tea can tolerate drought, frost, low pH, peat, shade, and slope. As a result of the long cultivation of tea, many cultivars have been developed that are based on flavor of the tea-producing substances, size of leaves and adaptability to climatic conditions. Named teas often depend on where they originate, the color produced or the combinations of tea that is blended. For example, Pekoe, Orange Pekoe and Flowery Pekoe names indicate partly the fineness of the leaf in each. Black teas that have undergone full fermentation or a chemical process, include Hyson, Young Hyson, Gunpowder and Imperial. Oolong Tea, a favorite in North America, comes from Taiwan and is partially fermented. Green teas come mainly from China, India and Sri Lanka.

While white tea is made up of premature tea leaves and buds picked at an early plant life stage and drying them, green tea is produced by harvesting mature leaves and gradually drying

the leaves; and black tea is produced by harvesting mature leaves and fermenting the leaves for the longest amount of time compared to white and green tea (Stangl, 2007).

In recent years, the health benefits of green tea have been widely publicized and extensively investigated due to its tea-derived polyphenols (Duffy et al., 2001; Yang et al., 2000). Polyphenols are abundant micronutrients in our diet (Manach et al., 2004), and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. 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 (Kai On Chua et al., 2004). These tea polyphenols include catechin (C), epicatechin (EC), and epigallocatechin gallate (EGCG).

The word ‗tea' is commonly used for anything infused in water to allow the water to obtain the flavor and aroma of the object that was steeped. Types of ‗faux-teas (meaning tea produced with anything other than leaves from *Camellia sinensis*)' include: chamomile, mint, rose, rooibos (a red bush native to Africa), and other plants used for infusion.

Limited research on white tea has been conducted, as it is a relatively new product in the Western hemisphere. Consequently, consumer friendly high performance liquid chromatography (HPLC) (Kai On Chua et al., 2004) research was conducted by writer on temperature and time variation on currently available and well-known Lipton® white tea to determine its polyphenol and methylxanthine concentrations. This was done to provide information for the most ideal preparation methods of white tea with respect to purported health benefits in corresponding antioxidant content.

A diet containing a balance of the various forms of antioxidants will maintain overall good health, and could even impact serious diseases. For instance, the American Cancer Society

encourages people to eat five to nine servings of fruits and vegetables per day and emphasizes the benefits of obtaining antioxidants through foods rather than supplements. The key is to eat a variety of fruits, vegetables, and nuts to ensure adequate intake from all the health benefits that antioxidants can provide (Barazesh, 2008).

Statement of the Problem

Polyphenols and methylxanthines were investigated in Lipton® White Tea with Blueberry and Pomegranate flavoring. Limited data on the quantifications of polyphenols and methylxanthines are available in commercial tea brands, specifically quantification of polyphenols and methylxanthine concentrations at different time and temperature conditions in white tea are scarce or unknown.

This tea was tested using reverse-phase HPLC to quantify the levels of catechin (C), epicatechin (EC), epigallocatechin-3 gallate (EGCG), and the methylxanthine, caffeine. The study using HPLC was conducted at University of Wisconsin-Stout Chemistry Department, third floor Jarvis Hall Science Wing in the spring season of 2008.

Purpose of the Study

Current research findings have revealed that green tea has properties that improve cognitive function (Kuriyama et al., 2006), improve running endurance (Murase et al., 2006), and decrease body fat composition while increasing one‘s energy expenditure (Dulloo et al., 1999). These findings point to various polyphenols in the tea as a causative factor for these beneficial results. The same polyphenols are found in white tea, and much attention is now focusing on suggesting that white may have greater polyphenolic concentrations than black or green tea; thus, making them more beneficial to the health of consumers. However, there is very little research to support this assumption. Therefore, the current research is designed to

determine the polyphenolic levels in Lipton® Blueberry and Pomegranate Flavored White tea. The findings will give credence to the hypothesis that white tea has beneficial qualities. Consumers will then be able to brew their white tea with successful efforts in obtaining the greatest concentrations of polyphenols and methylxanthines according to the most ideal steeping conditions supported by research findings.

Objectives of the Study

The first objective of this research was to determine the quantities of beneficial antioxidants per serving in a cup (250mL) of Lipton® White Tea with Blueberry and Pomegranate flavoring by comparing polyphenol and methylxanthine concentrations among varying brewing times of 2 minutes, 5 minutes, 10 minutes, and 24 hours and increasing temperatures (80°C, 85°C, 90°C, 95°C, and 100°C). The second objective was to compare the quantities of caffeine, catechin, epicatechin, and epigallocatechin gallate concentrations in white tea under the above mentioned varied time and temperature conditions. These findings will then be compared to previous findings (Gudala et al., 2008) from green tea. The research hypothesis was that there would be greater polyphenol and methylxanthine concentrations in white tea steeped in greater temperatures for longer periods of time.

The outcome expected was to determine the polyphenol concentrations in white tea given a specified steeping time and temperature. White tea manufacturers tend to recommend that the optimal white tea brewing temperature is 80-85 °C for approximately one minute (depending on the tea quality). This study‘s objectives and findings will be compared to current recommendations for tea steeping temperatures and duration. If higher brewing temperatures (100°C) than recommended are ideal for expressing greater concentrations, it may be beneficial

for the consumers to steep longer. This information would then be useful in achieving optimal health per increased antioxidant intake from tea.

Assumptions of the Study

In using Lipton® White Tea, there are a few assumptions that connect with this research. Younger tea leaves (of white tea) produce greater concentrations of polyphenol and methylxanthines than more mature leaves (green, oolong, and black teas) and that flavored and unflavored teas (each according to its kind) have equal concentrations of polyphenols and methylxanthines. Tea oxidation was not expected to occur during chemical analysis.

Definition of Terms

Polyphenol. Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets. Ranging from simple phenolic molecules to highly polymerized compounds with molecular weights of greater than 30,000 daltons, the occurrence of this complex group of substances in plant foods is extremely variable. Polyphenols traditionally have been considered antinutrients by animal nutritionists, because of the adverse effect of tannins, one type of polyphenol, on protein digestibility. However, recent interest in food phenolics has increased greatly, owing to their antioxidant capacity (free radical scavenging and metal chelating activities) and their possible beneficial implications in human health, such as in the treatment and prevention of cancer, cardiovascular disease, and other pathologies.

Catechin. Catechin and epicatechin are epimers, with (-)-epicatechin and (+)-catechin being the most common optical isomers found in nature. Catechin was first isolated from the plant extract catechu, from which it derives its name. Heating catechin past its point of

decomposition releases pyrocatechol (also called catechol), which explains the common origin of the names of these compounds.

Epigallocatechin gallate (EGCG). EGCG is the most abundant catechin in, most notably, tea, among other plants, and is also a potent antioxidant (Stangl et al., 2007) that may have therapeutic properties for many disorders including cancer (Duffy et al., 2001; Yang et al., 2000). It is found in white and green teas.

Methylxanthines. Methylxanthines are a diuretic agent (e.g., aminophylline, caffeine, or theophylline) that serves as a smooth muscle relaxant as well as a cardiac muscle and central nervous system (CNS) stimulant. Clinically, it is used as a bronchodilator to treat asthma symptoms. Caffeine was the main methylxanthine examined in this study.

Caffeine. Caffeine is the common name for 1,3,7-trimethylxanthine, a bitter substance found in coffee, tea, soft drinks, chocolate, some nuts and certain medications. When purified, caffeine produces an intensely bitter white powder that provides a distinctive taste in soft drinks. The word "caffeine" came from the German word *kaffee* and the French word *café*, each meaning coffee.

Antioxidant. This word is a combination of two, against and oxidants meaning a compound capable of neutralizing harmful compounds that invade our cells. Antioxidants come in several forms, including the vitamins A, C, and E; plant-derived polyphenols, found in colorful fruits and vegetables; and also the element selenium, found in nuts and broccoli (Barazesh, 2008).

These harmful molecules, known as free radicals, contain unpaired electrons—which is unusual because electrons typically come in pairs. Unpaired electrons make free radicals highly reactive, and in this state, they can cause damage by attacking the components of body cells, and

can even cause cancer (Barazesh, 2008),

Free radicals are created as a natural by-product of reactions in cells with exposure to iron causing oxidation in the body and causing an acidic environment (Barazesh, 2008). Other sources of free radicals include cigarette smoke, air pollution, and exposure to UV light or radiation. Once free radicals are formed, they can make more free radicals by scavenging electrons from other molecules.

Antioxidants neutralize free radicals either by providing the extra electron needed to make the pair, or by breaking down the free radical molecule to render it harmless. So, antioxidants stop the chain reaction of free radical formation and benefit human health by boosting the immune system. As antioxidants are used in the process of free radical neutralization, a diet rich in antioxidants is essential to ensure a constant supply.

Organization of Thesis

This thesis is organized into five chapters. Continuing from this first introductory chapter is the literature review (Chapter II) in which current research related to the topic of discussion is reviewed. The research methods are presented in Chapter III. Chapter IV reports the results, including a description of the tea used, HPLC function and uses, and procedures in graphical analysis of each experimental chromatogram. The final chapter, Chapter V, will discuss the study‘s findings and conclusions. Following the conclusions will be a short projection of these study findings‘ implications and recommendations for further research.

Chapter II: Literature Review

The popularity of tea has stood the test of time. Today, tea is enjoyed around the world and its consumption reflects local preference and tradition. For the past three decades, epidemiologists have observed lower risks of cancer, cardiovascular disease, and osteoporosis in populations that drink tea frequently (Hakim et al., 2001). This chapter will explore white tea's contents, growth characteristics, production, processing, and science-based health benefits.

History and Background

The history of tea is said it have started in China around 2750 BC. Chinese legends tells that an Emperor named Shen Nung was sitting in the shade of a wild tea tree, boiling a container of drinking water, when a breeze blew a few tea leaves into the boiling pot. The leaves gave the water a flavor that he found delicious. After experimenting further, he found it to have medicinal properties, as well as a appealing flavor. This emperor then motivated the people of China to plant tea bushes/trees for the benefit of the entire nation. It took 3,000 years for tea to become a popular drink throughout the Chinese empire. This emperor of old has come to be called, the Legendary Father of Tea, (Stash Tea Co., 2009).

Tea has also been taken for medicinal use. The infusion, once recommended in China as a cancer cure, contains some tannin, suspected of being anticarcinogenic. Chinese regard tea as an antitoxic, diuretic, expectorant, stimulant, and intestinal strengthening agent (Leung et al., 1996). Tea is often considered an oral astringent, stimulant, as well as a nerve sedative, and headache reliever. However, even with the above-mentioned benefits, it is reported to cause unpleasant nerve and digestive disturbances (Leung et al., 1996). According to Leung et al. (1996), tea is reportedly effective in the clinical treatment of various microbiological disorders including amebic dysentery, bacterial dysentery, gastroenteritis, and hepatitis. It has also been

reported to have antiatherosclerotic effects and bioflavanoid activity (Miura et al., 2001). Duke and Wain (1981) report that the plant has a folk reputation as analgesic, antidotal, astringent, cardiotonic, carminative, CNS-stimulant, demulcent, deobstruent, digestive, diuretic, expectorant, lactagogue, narcotic, nervine, refrigerant, stimulant, and stomachic; used for bruises, burns, cancer, cold, dogbite, dropsy, dysentery, epilepsy, eruptions, fever, headache, hemoptysis, hemorrhage, malaria, ophthalmia, smallpox, sores, toxemia, tumors, and even wounds throughout history.

The use of tea has been native to Southeast Asia, from Sri Lanka and India to Assam and China as tea has been planted widely in these and other tropical and subtropical areas. Near the Equator, it ranges up to nearly 2,000 meters above sea level elevation and is reported to tolerate annual precipitation of 7 to 31 decimeters with an annual temperature of 14 to 27°C, and pH of 4.5 to 7.3. Although evergreen, tea is resistant to frost, and requires equable, humid, warm situations; some Chinese tea varieties can tolerate cooler climates. Successful plantations have also been established in Charleston, North Carolina, United States. Figure 1 shows the tea plant (*Camellia sinensis)* in its natural form. These plants are found around the world that produces the beverage analyzed in this study.

Figure 1. Camellia sinensis illustration (Viklund, 2007)

Tea is propagated either from seeds or by seedling. Seedbearing trees, selected for yield and quality, are cross-fertilized, and the progeny of seed sown in new seed orchards are spaced 300-350 trees/ha. It requires 4-12 years to bear seed. Seeds from seed orchards are planted in a nursery or at a stake, protected from sun and wind (Duke, 1983).

The tea leaf has several uses. Dried and cured leaves are widely used as a beverage, which has a stimulant effect due to caffeine (Stash Tea Co, 2009). Steam distillation of black tea yields an essential oil. Tea extract is used as a flavor in alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, and puddings (Leung & Foster, 1996). Air-dry tea seed yields clear, golden-yellow oil resembling sasanqua oil containing saponin. Refined teaseed oil, made by removing the free fatty acids with caustic soda, then bleaching the oil with Fuller's earth and a sprinkling of bone black, makes an oil suitable for use in manufacture of sanctuary or signal oil for burning purposes, and in all respects is considered a favorable substitute for

rapeseed, olive, or lard oils (Leung & Foster, 1996). The oil is different from cottonseed, corn, or sesame oils, in that it is a non-drying oil and is not subject to oxidation changes, thus making it very suitable for use in the textile industry; it remains liquid below -18°C.

There are several different types of teas, differing according to their processing, and they include white, green, black, and oolong. White tea is made from drying young unrolled leaves and buds. Green tea is made from leaves steamed and dried, while black tea leaves are withered, rolled, fermented and dried. White teas buds and young leaves picked shortly before the buds have fully opened, which are steamed to inactivate polyphenol oxidase, and then dried. Thus, white tea retains the high concentrations of catechins (EGCG, C, and EC) present in fresh tea leaves (Santana-Rois et al., 2001). The tea takes its name from the silver fuzz that still covers the buds, which turns white when the tea is dried. The exact proportion of buds to leaves varies depending on the variety of white tea. For example, White Peony contains one bud for every two leaves, while Silver Needles, is made entirely from downy buds picked within a two-day period in early Spring (PR Log, 2009). Green tea is made from more mature tea leaves than white tea, and may be withered prior to steaming or firing. Although they are also rich in catechins, green teas may have different catechin profiles than white teas. Tea leaves destined to be sold as white tea undergo the least amount of processing compared to other tea leaves. Instead of air-drying, unwithered leaves are steamed. This is brewed to produce a pale tea with a sweet, silky flavor. People who have tried both note that white tea lacks the "grassy" aftertaste so often associated with green tea. In leaving tea leaves close to their natural state, white tea is believed to contain more polyphenols; those powerful anti-oxidants that fight and kill cancer-causing cells, than any other type of tea (Santana-Rios et al., 2001).

Freshly cut tea leaves consist of about 75% water (Cheyneir, 2005) as well as a variety of

tea flavors that are from the solid content of up to 25% (Figure 2). These tea flavors are formed through the combination of three main combinations: catechins (bitter and astringent), caffeine (bitter), theanine and amino acids (flavor and sweetness, respectively). Other components of green and white teas‘ solid matter include vitamins (A, B1, B2, B3, C) chlorophyll, various minerals, pectin, saccarides, and saponin (Teizer, 2005).

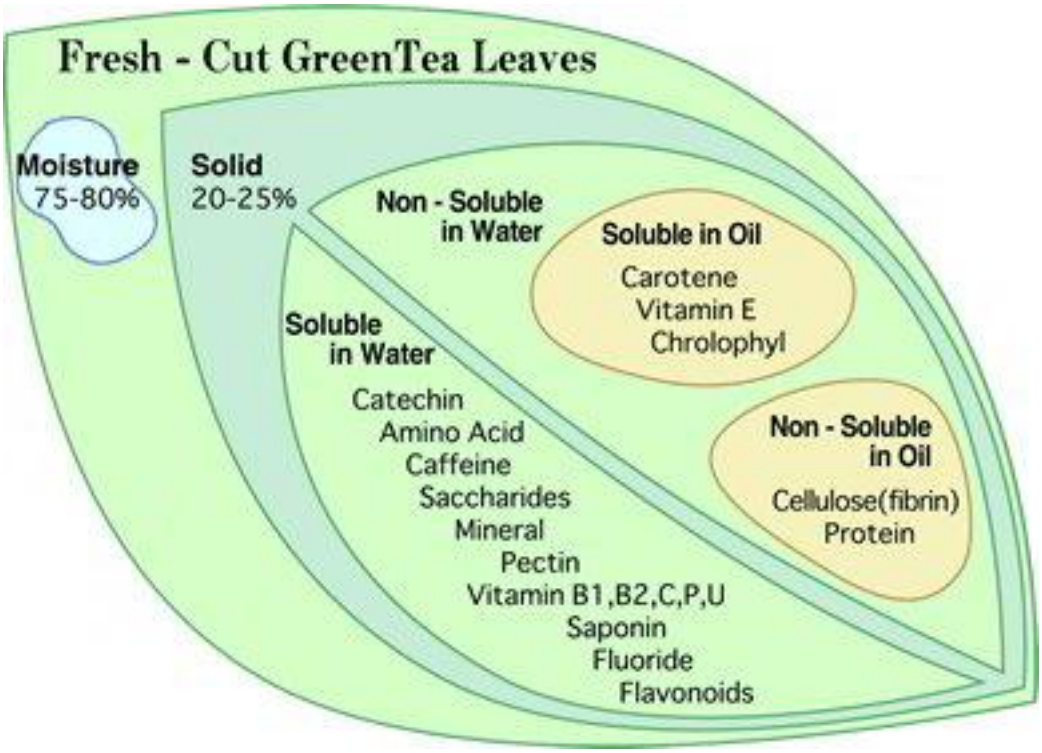

Figure 2. Composition of tea leaves (Graham, 1992)

Catechin (Figure 3), a tannin, is another chemical component that affects the taste of tea, which produces a bitter, astringent aroma, and is a powerful, water-soluble polyphenol and antioxidant that is easily oxidized. Catechin is one of these tannins, which makes up as much as 25% of the leaf‘s dry matter (Dulloo et al., 1999).

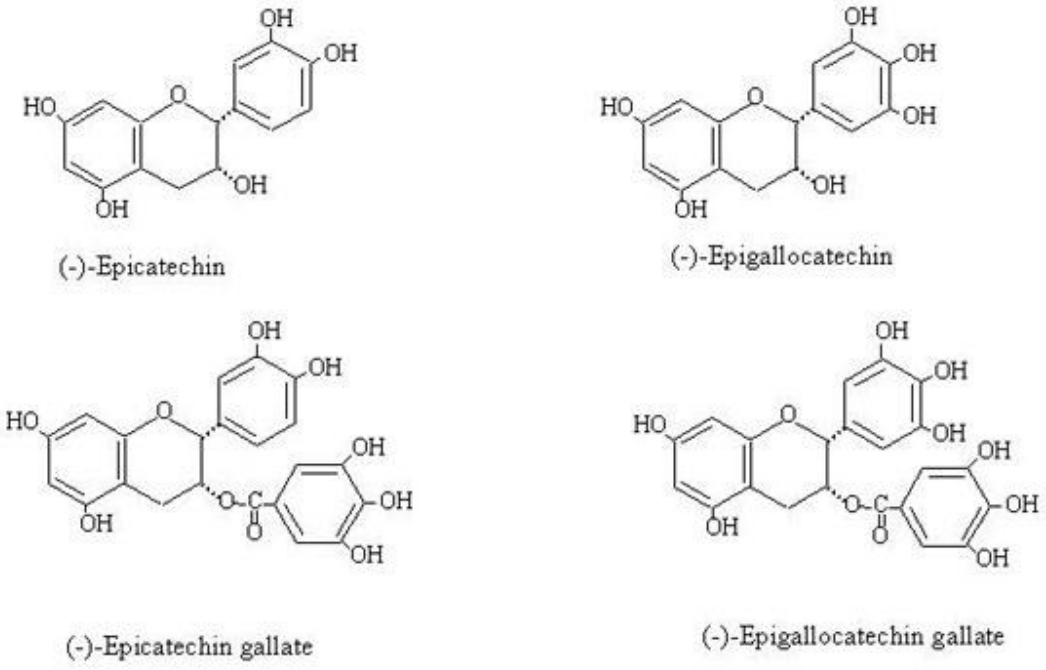

Figure 3. Polyphenol catechin chemical structures

Research aimed at elucidating the active compounds in tea has revealed that its protective effects are due primarily to catechins. Tea contains four main catechin substances: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigollocatechin gallate (EGCG) (Figure 3) all of which are inclusively called ‗catechin'. EGCG and caffeine are most abundant in white tea (Hilal & Engelhardt, 2007). The high antioxidant activity characteristic of tea makes it beneficial for protecting the body from oxidative damage due to free radicals. Research shows that green tea may help the arterial wall by reducing lipids (Anderson et al., 1998). Green tea can protect against experimentally induced DNA damage, and slow or halt the initiation and progression of undesirable cell colonies (Nakane, 1989). Various studies have shown evidence

that green tea may provide immuno-protective qualities, particularly in the case of patients undergoing radiation or chemotherapy. White blood cell count has appeared to be maintained more effectively in patients consuming five cups of green tea per day compared to non-supplemented patients (Pashad, 1998).

White tea is manufactured from fresh, unfermented tea leaves; the oxidation of catechins is minimal (Anderson et al., 1998), and hence they are able to serve as antioxidants. Researchers believe, according to Teizer (2005), that catechin is effective because it easily sticks to proteins, blocking bacteria from adhering to cell walls and disrupting their ability to destroy them. The catechin in green tea prevents viruses from adhering and causing harm. Catechin reacts with toxins created by harmful bacteria (many of which belong to the protein family) and harmful metals such as lead, mercury, chromium and cadmium.

Tannins in tea are a catechin and a key component in its taste providing astringency when consumed. The amount of catechin tends to increase as the season progresses (Teizer, 2005). Spring tea (first crop) contains 12-13% catechin (13-17% as tannin) while summer tea (third crop) contains 13-14% (17-21% as tannin). If leaf order is compared, younger leaves include more catechin than mature ones. First leaves contain 14%, second catechin leaves 13%, third 12%, and fourth leaves 12% (Cheyneir, 2005). This explains why second and third crop summer teas are more astringent while Bancha, a Japanese green tea, is less so. Gyokuro green tea, whose leaves are covered during growth, contains less catechin and astringency (10% as tannin) because it gets less sunshine then Sencha (Teizer, 2005).

Tea Quality and Growing Conditions

The highest-quality white teas are reportedly Silver Needle and White Peony, both of which have various grades (Teizer, 2005). Silver Needle is carefully hand selected from the

tender fleshy sprouts of the "Big White" or the "Narcissus" tea bush. If the buds are selected with two leaves intact, then the resulting selection will be made into White Peony tea. The leaves and other material left over from the selection of Silver Needle and White Peony will be processed into Noble, Long Life Eyebrow. Gong Mei is made from "chaicha" bushes and is processed slightly differently than other white teas.

The quality of white tea is greatly dependent on the season of harvesting. The best white tea is picked in early spring and is subject to numerous requirements. First of all, picking top-grade white tea is prohibited on rainy days or when the early morning dew is not dry. It should not be picked when the buds appear purple, when they are damaged by wind, people, or insects, when they have begun to open; when they are hollow, when they are too long or too thin, when there is one bud with three to four leaves, or when there is frost on the ground. White tea production is greatly dependent on the weather conditions when the tea is made (Teizer, 2005). Adjustments to the withering stage and the method of bake drying are determined by tea makers as they interpret the effect the weather will have on the withering process. Temperature and humidity of the environment will dictate the techniques and timing of the withering and bake drying process.

A tea maker's ability to balance solar and indoor withering of white tea is the major determining factor of quality. There are many nuances of white tea production that are dependent on the region and climate where the tea is made, but the major stages in the process are selective picking from specific varietals, withering, careful hand selection, and bake drying.

Processing

Processing is executed in a very precise way to yield a desired product. Terminal sprouts with 2-3 leaves are usually hand-plucked for green and black teas, 10 kg of green shoots (75-

80% water) produce about 2.5 kg dried tea (Anderson, 1998). Bushes are plucked every 7-15 days, depending on the development of the tender shoots. Figure 4 demonstrates the various ways to process the tea plant into different types of tea. These varied processing methods affect the chemical concentrations of catechins and methylxanines (Figure 4). Increased processing and fermentation (Figure 4) allow for the depletion of the concentration of total catechins in tea. This may also support claims that less processed tea has greater concentrations of antioxidants (Teizer, 2005).

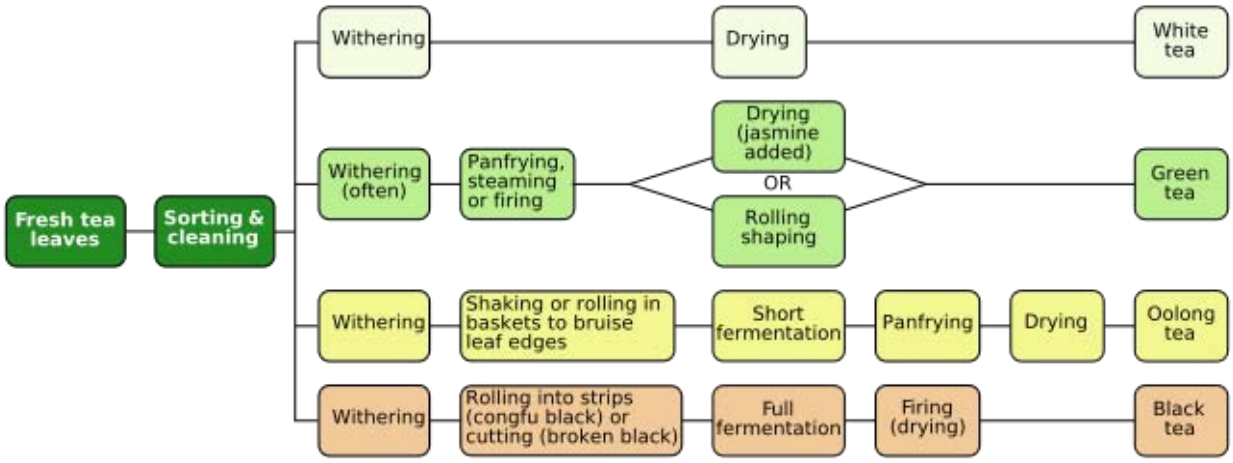

Figure 4. Camellia sinensis processing chart (Higdon, 2002.)

Black tea. Various techniques are used to produce black teas, usually during July and August when solar heat is most intense. Freshly picked leaves are spread very thinly and evenly on trays and placed in the sun until the leaves become very flaccid, requiring 13 hours or more, depending on heat and humidity. Other types of black teas are made by withering the leaves, rolling them into a ball and allowing them to ferment in a damp place for 3-6 hours, at which time the ball turns a yellowish copper color. If this stage goes too far, the leaves become sour and unfit for tea. After fermenting, the ball is broken up and the leaves spread out on trays and dried

in an oven until leaves are brittle and have slight distinct odor of tea. Tea leaves are then stored in air-tight tin boxes or cans.

Green Tea. As soon as harvested, leaves are steamed or heated to dry the natural sap and prevent oxidation. Still soft and pliable after the initial treatment, the leaves are then rolled and subjected to further firing. Once dried, the leaves are sorted into various grades of green tea.

White Tea. Tea buds and premature leaves are withered, sun dried, and sorted into their various grades of white tea. The production of white tea is different from other teas as this is the least processed of all teas and has the most delicate taste. White tea leaves come from a special varietal tea bush called Narcissus or chaicha bushes. The tea from this bush has little buds covered with silver hairs that give the young leaves a white appearance.

The unique nature of white tea's color, leaf shape and hair fragrance is mainly created during the withering stage. If mechanical drying is required the leaves are baked (not fired) at temperatures less that 40°C (Teizer, 2005). Only special ‗two leaves and a bud' are selected. The ideal is a leaf or two being wrapped around a newly developing shoot. These shoots are plucked and segregated from the rest of the leaf being plucked. These leaves are then naturally withered and the process on final manual selection occurs.

According to the different standards of picking and selecting, white teas can be classified as Yin Zhen Bai Hao (Silver Needle), Bai Mu Dan (White Peony), Gongmei (Tribute Eyebrow), and Shou Mei (Noble, Long Life Eyebrow) (Hilal & Engelhardt, 2007). All of these white teas are widely produced in China. The highest-quality white teas are reportedly Silver Needle and White Peony, both of which have various grades and are primarily produced in the Fuding and Zhenhe districts of Fujian, China. Silver Needle is carefully hand selected from the tender fleshy sprouts of the "Narcissus" tea bush. If the buds are selected with two leaves intact, then the

resulting selection will be made into White Peony tea. The leaves and other material left over from the selection of Silver Needle and White Peony will be processed into Noble, Long Life Eyebrow.

Figure 5 illustrates the effects of processing on tea polyphenol concentrations. It has been explained above that *Camellia sinensis* can undergo a variety of processes to achieve different products. White tea, being the least processed of the teas, has the largest concentration of catechins as compared to the more processed teas. Theaflavins and thearubigins increase in concentration as processing procedure increases, so that their greatest concentrations are in black teas. They are formed enzymatically during the fermentation or enzymatic oxidation of the tea leaves (Robertson & Bendall, 1983).

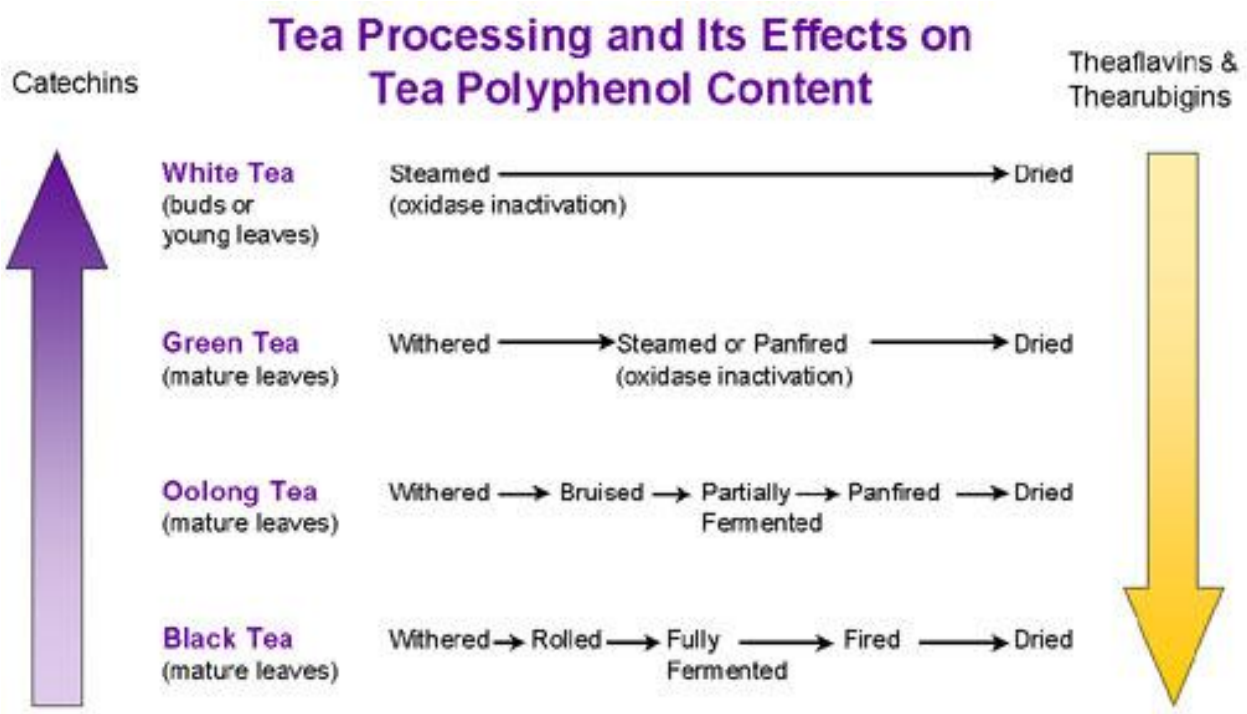

Figure 5. General processing effects on tea‘s polyphenol concentration (Teizer, 2005)

Health benefits

Numerous research studies show promising effects on tea‘s antibacterial, anti-inflammatory, protection against cancers and cell line growth, blood glucose lowering, brain health, cholesterol lowering properties, hypertension, weight loss, and many more.

Antibacterial. A particularly exciting discovery (Sanaka et al., 1996) related to the antibacterial properties of green tea polyphenols shows that these compounds inhibit the growth and adherence of oral bacteria particularly periodontal-causing bacterium, *Porphyromonas*, and decay-causing bacteria such as *Streptococcus salivarius* and *Streptococcus mutans.* A supporting study (Rasheed & Haider, 1998), confirms that *Streptococcus mutans* are inhibited completely by contact with green tea polyphenols. Effects of polyphenolic compounds isolated from green tea on the growth and adherence of Porphyromonas gingivalis onto human buccal epilthelial cells were investigated by Sanaka et al., 1996. Green tea polyphenols completely inhibited the growth and adherence of P. gingivalis onto the buccai epithelial cells at concentrations of 250-500 micrograms/mL. One possible mechanism of the action of tea in preventing dental decay is its ability to inhibit the enzyme amylase present in the saliva. Thus, less starch gets converted in the mouth into bacteria-feeding simple sugars such as glucose and maltose. Bacterial amylase is likewise inhibited, making less nutrition available to the decay-causing organisms.

Green tea catechins also help destroy harmful intestinal bacteria. It was noted in one study that when tube-fed patients received 300 mg of tea catechins a day, the putrefactive products in their gastrointestinal tract decreased, and organic acids increased, lowering the pH (Sanaka et al., 1996). The greater acidity is highly beneficial, since it makes the environment inhospitable to harmful bacteria, while beneficial lactic acid bacteria can thrive. Indeed, the bactericidal activity of green tea does not affect lactic acid bacteria. Decreased levels of

putrefactive products and improved intestinal flora lead to better digestion, better immune function, and lower risk of colorectal cancer.

Brain Health. Alzheimer‘s disease (AD) is a greatly distressing disease characterized by the extracellular evidence of beta-amyloid peptide (Abeta) in cerebral plaques. Abeta is derived from the beta-amyloid precursor protein (APP) by enzymes alpha-, beta- and gamma-secretase. Compounds that enhance alpha-secretase, but inhibit beta- or gamms-secretase activity, have therapeutic potential in the treatment of AD. Green tea or its major polyphenolic compound (EGCG), has been shown to have neuroprotective effects.

A study (Lee et al., 2009) investigated the possible effects of EGCG on memory dysfunction related to Abeta-inducement of secretase activities in AD. Mice were pretreated with EGCG (1.5 or 3 mg/kg body weight in drinking water) for three weeks before intracerebro-ventricular administration of 0.5 micrograms Abeta (1-42). The Abeta (1-42) induces a decrease in brain alpha-secretase and increases in both brain beta- an gamma secretase activities in the cortex and hippocampus, which were significantly reduced by EGCG by 54%. To further test the ability of EGCG to affect memory, EGCG (3mg/kg body weight) was given in drinking water for one week to genetically developed preseniline 2 (PS2) mutant AD mice. In comparison to the untreated mutant PS2 AD mice, treatment with EGCG enhanced memory function and inhibited the fibrillization of Abeta *in vitro* with half maximal inhibitory concentration of 7.5mg/L. These findings may be beneficial in the prevention of development or progression of AD.

Cholesterol. Research shows (Lin et al., 1998) that green tea lowers total cholesterol and raises HDL ("good") cholesterol in both animals and people. When rats were fed 2.5% green tea leaves in their diet the experimental group showed a drop in total cholesterol, low-density

cholesterol, and triglycerides. The body weight of green tea-fed rats was 10 to 18% lower than that of rats not consuming green tea. In addition, the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase, and anti-carcinogenic phase-II enzyme glutathione S-transferase (GST), were significantly greater in the green tea group, as was the glutathione level in the liver. There was no liver or kidney toxicity. Thus, the study demonstrated combined cardiovascular and anticancer effects of green tea. Results from other animal investigations suggest that polyphenols in green tea may block the intestinal absorption of cholesterol and promote its excretion from the body. This would result in a decreased lipid absorption in the body.

Additionally, in a double-blind, randomized, placebo controlled trial (Maron et al., 2003), 240 adults were given either theaflavin-enriched green tea extract in form of 375mg capsule daily or a placebo. After 12 weeks, patients in the tea extract group had significantly less low-density lipoprotein cholesterol (LDL-C) and total cholesterol (16.4% and 11.3% lower than baseline, respectively, $p<0.01$) compared to the placebo group. It was concluded that the theaflavin-enriched green tea extract can be used together with other dietary approaches to reduce LDL-cholesterol (Maron et al., 2003). This conclusion was supported in other studies conducted and documented (Yang & Koo, 1997).

Diabetes. Diabetes is an illness characterized by the overabundance of glucose molecules in the blood. High glucose concentration over time can induce retinopathy, neuropathy, non-healing ulcers, and other physiological difficulties (Tierney et al., 2004). Tea polyphenols have demonstrated the ability to lower serum glucose in humans in that tea polyphenols inhibit the activity of amylase, a starch-digesting enzyme found in saliva and in the intestines (Zhang & Kashket, 1998). In this study, researchers found that, consuming tea results in a delayed decomposition of starch and the serum glucose of a person who has diabetes is

minimized by 73.5%. The main mechanism is the inhibition of the activity of starch digesting enzyme amylase. Tea inhibits both salivary and intestinal amylase, so that starch is broken down more slowly, so, the rise in serum glucose is thus minimized.

A different study (Bryans et al., 2007) investigated the effect of consuming instant black tea on postprandial plasma glucose and insulin concentrations in healthy humans in response to an oral glucose load. A four-way randomized, crossover trial was designed in which 16 healthy fasted subjects would consume 75g of glucose in either 250ml of water (control), 250ml of water plus 0.052g of caffeine (positive control) or 250 ml of water plus 1.0g or 3.0g of instant black tea. Blood samples were collected at fasting and at 30-minute intervals for 150 minutes from commencement of drink ingestion. Glucose and insulin concentrations were measured using standard methodology. The tea was chemically characterized using colorimetric and HPLC methods.

Chemical analysis showed that the tea was rich in polyphenolic compounds (total, 350mg/g). Results from only 3 treatment arms are reported because the 3.0g tea drink caused gastrointestinal symptoms. Plasma glucose concentrations <60min in response to the drinks were similar, but were significantly reduced at 120min ($P<0.01$), following ingestion of the 1.0g tea drink, relative to the control and caffeine drinks. Tea consumption resulted in elevated insulin concentrations compared with the control and caffeine drinks at 90 minutes ($P<0.01$) and compared with caffeine drink alone at 150 minutes ($P<0.01$).

The 1.0g tea drink reduced the late phase plasma glucose response in healthy humans with a corresponding increase in insulin. This may indicate that the attenuation in postprandial glycemia was achieved as a result of an elevated insulin response following stimulation of pancreatic ß-cells. This effect may be attributable to the presence of phenolic compounds in the

tea. It may also assist in decreasing the insulin response in people who have diabetes and the harmful effects that can result from the illness.

Hypertension. Hypertension is one of the most common forms of CVD, affecting millions of people throughout the world and about 20% of the adult population in many countries. It is interrelated with coronary artery disease, stroke, congestive heart failure, and renal dysfunction, and is one of the major risk factors for cardiovascular-related mortality, which accounts for 20-50% of all deaths (Hypertension control, 1996).

One study (Yang et al., 2004) examined the effect of tea drinking, measured in detail on the risk of newly diagnosed hypertension in 1,507 subjects (711 men and 796 women), 20 years or older, who did not have a hypertensive history during 1996 in Taiwan. Results showed that 600 subjects (39.8%) were habitual tea drinkers, defined by tea consumption of 120 mL/day or more during the past year. In comparison to non-habitual tea drinkers, the risk of developing hypertension was 46% less for those who drank 120 to 599 mL/day and was further reduced by 65% for those who drank 600 mL/day or more after carefully adjusting for age, sex, socioeconomic status, family history of hypertension, body mass index, waist-hip ratio, lifestyle factors (total physical activity, high sodium intake, cigarette smoking, alcohol consumption, and coffee drinking), and dietary factors (vegetable, fruit, unrefined grain, fish, milk, visible-fat food, and deep fried food intake). However, tea consumption for more than 1 year was not associated with a further reduction of hypertension risk. Yang and his associates concluded that there was significant evidence to show that habitual moderate strength green or oolong tea consumption, 120 mL/day or more for 1 year, significantly reduces the risk of developing hypertension in the Chinese population.

Anticarcinogenic. Cancer is a disease caused by increased proliferation of cells which group and form a lump called tumor. Tumors can be benign or malignant. Cells from malignant tumors break away from the original tumor and spread to other parts of the body growing and forming new tumors. They can invade, penetrate into blood and lymphatic vessels, circulate via the bloodstream and can grow in a normal organ or tissue anywhere in the body. Unfortunately, treatment options for metastasis are very limited and usually represent the end stage of the disease. Unlike malignant tumors, benign tumors do not invade and, with very rare exceptions, are not life threatening.

Cancer is also serious health problem and cause of global concern. Experimental studies have demonstrated the inhibitory effect of tea infusions and its components, specifically, polyphenols on chemical carcinogenesis of various cancers in experimental animals. The chemopreventive effects of green tea depend on: its antioxidant action (Graham, 1992); specific induction of detoxifying enzymes (Mekay et al., 2002); its molecular regulatory functions on cellular growth (Balentine et al., 2000), development and apoptosis; and selective improvement in function of intestinal bacterial flora (Carmen & Reyes, 2006). An important aspect of cancer risk is related to inflammatory response; currently, anti-inflammatory agents are used in chemopreventive strategies. The inflammatory response involves production of cytokines and proinflammatory oxidants such as hypochlorous acid and peroxynitrite produced by neutrophils. Green tea catechins and soy isoflavones have also been shown to be chemopreventive. The aromatic nature of polyphenols makes them potential targets of hypochlorous acid and peroxynitrite, and these reactions may create novel pharmacophores at the site of inflammation. In addition, a major mechanism of the anticarcinogenic activity of green tea in animals is impairment of interaction of carcinogens with DNA that could ultimately lead to mutations.

Quinol oxidase (NOX) is an enzyme required for growth by both normal and malignant cells. While normal cells express NOX only when dividing, tumor cells express it all the time. The tumor form of the enzyme is called t-NOX, or tumor-associated NOX. Drugs that inhibit tNOX also inhibit tumor growth. Green tea lowers serum glucose and consequently insulin. Since elevated insulin is a potent growth factor for many kinds of tumors, as well as a pro-inflammatory and immunosuppressive hormone, the lowering of insulin in a tumor should help prevent cancer or, in cases of existing cancer, slow down its growth.

Green and black tea polyphenols have been shown to initiate in the growth inhibition of many cell lines; some founded results are summarized in Table 1. The efficacy of inhibition varied, depending on the cell line used. EGCG was generally the best inhibitor in most of the cell lines tested, with 50% inhibition (IC_{50}) values ranging between 22 and 130μmol/L. EGC and GC were better inhibitors toward A427 cells with IC_{50} values of 34 and 38μmol/L, respectively. The growth inhibition of Ha-ras-transformed 21 BES cells by the black tea polyphenol, theaflavin-3_-digallate, was similar to the growth inhibition caused by green tea polyphenols, EGCG and EGC (Yang et al., 1998). These studies demonstrate the biological activities of tea polyphenols, but the effective concentrations observed are generally 1-2 orders of magnitude higher than peak human plasma concentrations. The lowest effective concentration of EGCG (1-2μg/L) resulted in the inhibition of the transformation of preneoplastic human mammary epithelial cells by benzo[a]pyrene (Katdare et al., 1998).

Tea polyphenols may inhibit cell growth through a variety of mechanisms. One mechanism is through apoptosis. Human cancer cell lines that have shown apoptosis-like changes after EGCG or EGC treatment, at levels of 86-2000μmol/L, include PC-9, H661, KATO III, DU 145, A431, HaCat, and Molt043 (Table 1) (Ahmed et al., 1997; Hibasami et al., 1996

and 1998; Okabe et al., 1997; Yang et al., 1998). EGCG may also act as a prooxidant through H_2O_2 production to include apoptosis. Addition of catalase into the culture media inhibited EGCG-induced apoptosis (Yang et al., 1998). In combination with other chemopreventative drugs such as sulindac and tamoxifen, EGCG induced a synergistic apoptotic effect (Suganuma et al., 1999). Other mechanisms for the growth inhibition of cancer cells may be through the induction of cell cycle arrest by EGCG and the inhibition of signal transduction pathways leading to the activation of important transcription factors activator protein 1 (AP-1) and nuclear factor κB (NF-κB) (Ahmad et al., 1997; Dong et al., 1997; Lin and Lin, 1997; Okabe et al., 1997).

Table 1.

Growth inhibition and apoptosis caused by tea polyphenols in human cancer cell lines

Human cancer	Cell line	Biological activity	EC_{50}[1] EGCG, μmol/L	Reference
Human oral	1483 HNSCC	Growth inhibition	18	(Khafif et al., 1998)
Human breast	MCF-7	Growth inhibition	120	(Valcic et al., 1996)
Human lung	PC-9	Growth inhibition	140	(Suganuma et al., 1997)
		Apoptosis	100	(Okabe et al., 1997)
	A-427	Growth inhibition	94	(Valcic et al., 1996)
	H441	Growth inhibition	60	(Yang et al., 1998)
	H661	Growth inhibition	22	(Yang et al., 1998)
		Apoptosis	100	
	H1299	Growth inhibition	22	(Yang et al., 1998)
Human stomach	KATO III	Apoptosis	2000	(Hibasami et al., 1996)

Human colon	Caco-2	Growth inhibition	40	(Chen et al., 1998)
	HT-29	Growth inhibition	86	(Valcic et al., 1996, Yang et al., 1998)
Human prostate	DU145	Apoptosis	175	(Ahmad et al., 1997)
Human skin	A431	Apoptosis	87	(Ahmad et al., 1997)
	HaCat	Apoptosis	175	(Ahmad et al., 1997)
	UACC-375	Growth inhibition	130	(Valcic et al., 1996)
Human blood	Molt-43	Growth inhibition	100	(Hibasami et al., 1996)
		Apoptosis	100	

[1] Effective concentrations of EGCG, used for 50% or greater activity, are provided as examples. Concentrations of other tea polyphenols can be found in some of the references.

Cancer Cell lines. Tea polyphenols generally tend to have anti-cancer effects that result in growth inhibition of cell lines of many different types of cancers. Some examples are summarized in Table 1. Apoptosis is a form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area. It plays a crucial role in developing and maintaining health by eliminating old cells, unnecessary cells, and unhealthy cells. EGCG was the best overall inhibitor in most of the cell lines tested with 50% inhibition values ranging between 22-130 μmol/L. These studies demonstrate the biological activities of tea polyphenols, but the effective concentrations observed were generally higher than peak human plasma concentrations. The lowest effective concentration of EGCG (1-2μg/L) was observed in the inhibition of the transformation of preneoplastic human mammary epithelial cells by benzo(a)pyrene (Lang et al., 2000). So, this study supports the theory that 10 cups (2,500mL) of green tea/day would be needed to effectively result in lowering cancer risks.

Colo-rectal Cancer. Several researchers found that green tea constituent epigallocatechin-3-gallate inhibits topoisomerase I activity in human colon carcinoma cells (Berger et al., 2001). DNA topoisomerases I and II are essential for cell survival and play critical roles in DNA metabolism and structure. Inhibitors of topoisomerase constitute a novel family of antitumor agents with demonstrated clinical activity in human malignancies. The clinical use of these agents is limited due to severe toxic effects on normal cells. Therefore, there is a need to develop novel, nontoxic topoisomerase inhibitors that have the ability to spare normal cells. Recent studies have shown that green tea and its major polyphenolic constituent, epigallocatechin-3-gallate (EGCG) impart growth inhibitory responses to cancer cells but not to normal cells. Based on the knowledge that EGCG induces DNA damage of cancer cells, cancer cell cycle arrest, and apoptosis, the researchers considered the possibility of the involvement of topoisomerase in the antiproliferative response of EGCG. Therefore, for the first time, it has been shown that EGCG inhibits topoisomerase I, but not topoisomerase II in several human colon carcinoma cell lines. Based on this study it is tempting to suggest that combination of EGCG with other conventional topoisomerase inhibitors could be an improved strategy for treatment of colon cancer. The above-mentioned concluded the role of EGCG as a chemotherapeutic agent needed to be further investigated.

Esophageal Cancer. Experimental studies have shown that tea and tea polyphenols have anticarcinogenic properties. In one study, a nested case-control design was used to investigate the association between prediagnostic urinary tea polyphenol markers and subsequent risk of gastric and esophageal cancers (Sun et al., 2002). One hundred and ninety incident cases of gastric cancer and 42 cases of esophageal cancer

occurring in members of the Shanghai Cohort (18,244 men aged 45-64 years at recruitment with up to 12 years of follow-up) were compared with 772 cohort control subjects. After exclusion of cases diagnosed under 4 years follow-up, urinary EGC positivity showed a statistically significant ($P<0.01$) inverse association with gastric cancer was found. The protective effect was primarily seen among subjects with low (below population median) serum carotenes. Similar tea polyphenol-cancer risk associations were observed when the gastric cancer and esophageal cancer sites were combined. The study provides direct evidence that tea polyphenols may act as chemopreventive agents against gastric and esophageal cancer development.

Another study (Yang et al., 1999) suggested that EGCG was converted to EGC in the oral cavity, and both catechins were absorbed through the oral mucosa through drinking green tea rather than using green tea extracts. Because of the possible application of tea in the prevention of oral and esophageal cancers, the salivary levels of tea catechins were determined in six human volunteers after drinking tea preparations (equivalent to 2-3 cups of green tea). Saliva samples were collected after thoroughly rinsing the mouth with water. After drinking green tea preparations equivalent to two to three cups of tea, peak saliva levels of (-)-epigallocatechin (EGC; 11.7-43.9 microg/ml), EGC-3-gallate (EGCG; 4.8-22 microg/ml), and (-)-epicatechin (EC; 1.8-7.5 microg/ml) were observed after a few minutes. These levels were 2 orders of magnitude higher than those in the plasma. Taking tea solids in capsules resulted in no detectable salivary catechin level. Holding an EGCG solution in the mouth for a few minutes resulted in EGCG and EGC in the saliva and, subsequently, EGC in the urine. The present results

suggest that slowly drinking tea may be a very effective way of delivering large concentrations of catechins to the oral cavity and the esophagus.

Weight Loss. An increase in metabolism, may aid in weight loss (Dulloo et al., 1999) which is directly related to a 16 kDalton protein hormone that plays a key role in regulating energy intake and energy expenditure called leptin. Leptin is produced by fat cells that appear to play an important role in how the body manages fat storage through brain signals. Years ago, it was thought by scientists that lower leptin levels would increase appetite, however, current research has now found that it does just the opposite and decreases appetite. There is clear evidence that green tea's polyphenols (EGCG) are a factor in depressing leptin as well as affecting other hormone levels important in regulating appetite.

Green tea holds promise in many areas of weight loss. Besides affecting leptin levels, green tea also increases noradrenaline levels. Noradrenaline is a chemical neurotransmitter in the nervous system that plays a major role in activation of brown fat tissue. Activation of brown fat by increased noradrenaline levels is significant because it burns calories from the white fat located around a human waistline, hips and thighs. In one study (Duloo et al., 1999), it was found that green tea extract resulted in a significant increase in energy expenditure (a measure of metabolism). It also had a significant effect on fat oxidation (103 ± 13g, $P<0.05$) in a 24-hour period in the experimentally controlled respiratory chambers. While some of the effects were originally theorized to be due to the caffeine content of green tea, researchers discovered that green tea extract has properties that go beyond those that would be explained by caffeine alone.

Green tea also appears to increase energy expenditure related to fat oxidation. The green tea extract may play a role in the control of body composition and weight maintenance. Researchers studied the effects of green tea on 10 healthy young men, average age 25, who

ranged from lean to mildly overweight. For 6 weeks, the men took two capsules at each meal: green tea extract plus 50 milligrams of caffeine; 50 milligrams of caffeine; or a placebo (inactive capsule) (Teizer, 2005).

The study participants were on a weight maintenance diet of about 13% protein, 40% fat, and 47% carbohydrates, a "typical Western diet." Three times during the study, the men spent 24 hours in a testing room where investigators measured participant respiration and energy expenditure. Energy expenditure, the number of calories used during a 24-hour period, was higher for men taking green tea extract than for those taking caffeine or placebo. They also found evidence that men taking the green tea extract used more fat calories than those taking the placebo.

There was no difference between the caffeine users and the placebo users in terms of either overall calorie burning or fat calorie burning. The researchers therefore concluded that the increased calorie burning in the green tea group cannot be explained by caffeine intake alone. The investigators suggest that the caffeine interacted with natural substances in green tea called flavonoids to alter the body's use of norepinephrine, a chemical transmitter in the nervous system, and increased the rate of calorie burning. The researchers point out that, unlike some diet products, green tea does not contain high doses of caffeine, and it did not affect the heart rate in the study participants (Dulloo et al., 1999).

The researchers indicated that their findings have substantial implications for weight control. A 4% overall increase in 24-hour energy expenditure was attributed to the green tea extract, however, the research found that the extra expenditure took place during the daytime. This led them to conclude that, since thermogenesis (the body's own rate of burning calories) contributes 8-10% of daily energy expenditure in a typical subject, that this 4% overall increase

in energy expenditure due to the green tea actually translated to a 35-43% increase in daytime thermogenesis (Dulloo et al., 1999).

Of critical importance to thyroid patients is the fact that none of the research subjects reported any side effects, and no significant differences in heart rates were noticed. In this respect, green tea extract is different from some of the prescription drugs for obesity, and herbal products such as ephedra, which can raise heart rates and blood pressure, and are not recommended for many individuals, in particular, those with thyroid disease who may be particularly sensitive to stimulant (Dulloo et al., 1999).

The study mentioned above expresses that teas, green tea‘s EGCG especially, may have constituents useful in preventing and treating human illnesses and disease. The health benefits of teas featured above express a great need for further studies and research in confirming and expanding on the knowledge of white tea‘s health implications. This current study could therefore provide consumers information on polyphenol and methylxanthine concentrations present in each steeping condition; as this study is focused on the effect of varied extraction parameters on the polyphenols and methylxanthines of white tea commercially available in the United States.

Chapter III: Methodology

Chapter three includes a description of the methods taken for tea sampling, peak detection, and analysis used in this study as well as the study‘s instrumentation and limitations.

Polyphenols such as catechin (C), epicatechin (EC), and epigallocatechin gallate (EGCG), and the methylxanthine caffeine are unique as they all have similar polarities on UV spectra. Therefore, they are most efficiently simultaneously extracted and analyzed via high performance liquid chromatography (HPLC). Chua et al. (2004) reports HPLC as the preferred instrumentation as it offers extractions to be taken at multiple wavelengths recorded on a computer database.

HPLC is a form of column chromatography used to separate, identify, and quantify compounds based on like-polarities and stationary phase interactions with the column. Rather than gravity, a pump within the HPLC system provides the high pressure required to propel a mobile phase and analytes through a densely packed column. This density increases linear velocity resulting in accurate resolution in the resulting chromatogram. The type of chromatography used was reverse-phase chromatography.

Reverse-phase chromatography is a partitioning mechanism to affect separation. Separation takes place in the column where the stationary phase (non-polar), the mobile phase (polar), and the sample components interact. The majority of reversed phase separation is performed in several steps.

The first step in the chromatographic process is to equilibrate the column packed with the reverse phase medium. The polarity of the mobile phase is controlled by adding acetonitrile, an organic modifier (used in mobile phase A). In all cases, the polarity of the initial mobile phase

must be low enough to dissolve the partially hydrophobic solute yet high enough to ensure binding of the solute to the reverse phase chromatographic matrix.

In the second step, the sample containing the solutes to be separated is applied. Ideally, the sample is dissolved in the same mobile phase used to equilibrate the chromatographic bed. In the experimentation, white tea was injected into the column at a flow rate where optimum binding occurred (1.0mL/min). After the sample was applied, the chromatographic bed was washed further with mobile phase A in order to remove any unbound solute molecules.

In the third step, bound solutes are desorbed from the reverse phase medium by adjusting the polarity of the mobile phase so that the bound solute molecules desorb and elute from the column. In reverse phase chromatography, this usually involves decreasing the polarity of the mobile phase by increasing the percentage of organic modifiers in the mobile phase. This is accomplished by maintaining a high concentration of organic modifier in the final mobile phase (mobile phase B). The gradual decrease in mobile phase polarity (increasing mobile phase hydrophobicity) is achieved by an increasing linear gradient from 100% initial mobile phase A containing little or no organic modifier to 100% mobile phase B containing a higher concentration of organic modifier. The bound solutes desorb from the reversed phase medium according to their individual hydrophobicities.

The fourth step in the reverse phase process involves removing substances not previously desorbed. This is generally accomplished by changing mobile phase B to near 100% organic modifier in order to ensure complete removal of all bound substances prior to re-using the column.

The fifth step is re-equilibration of the chromatographic medium from 100% mobile phase B back to the initial mobile phase conditions.

Therefore in general, typically a reversed phase separation of molecules is individually achieved using a broad range gradient from 100% mobile phase A to 100% mobile phase B. The amount of organic modifier in both the initial and final mobile phases can also vary greatly. However, routine percentages of organic modifier are 5% or less in mobile phase A and 95% or more in mobile phase B.

Separated components of the tea sample were detected and ciphered by a UV light beam passed through the analyte flow cell after column separation at different absorbances (260, 270, 280, 290μm).

All methods were completed following previous procedures (Kafley, 2008). To detect polyphenols and methylxanthines, this experimentation utilized HPLC instrumentation with two pumps, a solvent programmer, autosampler, automatic injector, photodiode-array detector, and a Waters© computer data analysis system (Milford, MA). HPLC separates liquids into its components, so that each component is shown as a narrow band or peak. The time that it takes for a peak to exit the column determines the component species and the peak height or peak area determines the component‘s quantity.

Sample Selection

Commercially available Lipton® White Tea with Blueberry and Pomegranate flavoring was purchased over the internet from the producing company‘s website.

Standard Solution Preparation

Caffeine stock solutions were prepared as follows (Table 2) by massing 100mg of caffeine into 500mL methanol and 500mL Milli-Q® water. The mixture was heated to 60°C to dissolve the anhydrous solid. After the mixture was cooled to room temperature, it was then diluted with 50:50 (v/v) methanol, Milli-Q® water.

Catechin and epicatechin stock solutions were prepared by massing 100mg catechin and epicatechin in separate beakers. The mixtures were then dissolved in 50mL methanol and diluted to 100mL with Milli-Q® water.

Stock solutions were transferred via pipet into 100-mL volumetric flasks, then diluted to volume with methanol. All compounds were mixed together to prepare individual standard solutions in order to determine a standard retention time of each compound. Table 2 contains the five different standard solutions and concentrations in increasing amounts.

Table 2.

Standard mixture concentrations

Standard Element	Concentration (mg/L)				
	I	II	III	IV	V
Catechin	10	20	30	40	50
Epicatechin	20	40	60	80	100
Epigallocatechin Gallate	20	40	60	80	100
Caffeine	40	80	120	160	200

Final standards were prepared by measuring 1, 2, 3, 4, and 5 mg (respectively) of EGCG in separate 25mL volumetric flasks. Then the first flask was diluted to mark with standard mix #1, the second to mark with standard mix #2, the third to mark with standard mix #3, the fourth to mark with standard mix #4, and the fifth to mark with standard mix #5.

Reagents needed for the mobile phase in the HPLC included HPLC grade acetonitrile (CH_3CN)/0.25% glacial acetic acid (40%/60%; v/v), B phase, and 0.5% glacial acetic acid, A

phase, from Aldrich (Milwaukee, WI). The flow rate for phase A was set for 2 mL/minute. Phase B's flow rate was set at 0.0mL/minute.

Tea sample preparation

All samples were run in triplicate. Chippewa Spring water (250mL) was heated in a 400mL beaker. After the water reached an initial steeping temperature of 100°C, 95°C, 90°C, 85°C, 80°C, it was removed from the heat and poured over a commercially manufactured bag of Lipton© White Tea with Blueberry and Pomegranate flavoring (average mass was 1.75g) in a room temperature (25°C) 400mL beaker. Each of the triplicate samples were steeped in time intervals of 2 minutes, 5 minutes, 10 minutes, and 24 hours (using 60 tea samples for tea experimentation). The tea was then allowed to steep at room temperature at specific time intervals. As time intervals lapsed, each tea bag was removed from its beaker and excess water from the tea bag was expelled into the beaker of tea. Small tea samples of each triplicate were then transferred and filtered via 0.45μm syringe-mounted membrane filter (Stricher, 1993) into a 1mL autosampler vial for subsequent HPLC injection and analysis.

Instrumentation

A Waters© high performance liquid chromatography (HPLC) system with Millennium© software was used to identify and quantify catechin, epicatechin, epigallocatechin gallate, and caffeine in the tea samples from a modified HPLC method (Yang, 1998). The column used in the analysis was a Waters Radial Compression© 10 cm x 8 mm ID Novapak C_{18} column with a NovaPak GaurdPak in an RCM-100 radial compression module. Solvent A consisted of Milli-Q© water/glacial acetic acid 99.5:0.5 (v/v) and solvent B consisted of Milli-Q© water/acetonitrile/glacial acetic acid 59.5:40.0:0.5 (v/v). The solvent condition was changed from 100% A to 100% B within a 30 minute time range. The

solvent was then returned to 100% A for one minute, then equilibrated for the remaining 5 minutes. Gradient conditions for the complete 36-minute extraction are described in Table 3. The injection volume for each sample was 25µL. The HPLC hardware used included a Waters© 717 Plus autosampler, Waters© 1525 Binary HPLC Pump, and a Waters© Photodiode Array Detector. The system was controlled via PC using a Windows© NT operating system and Waters Empower© 2.0 software.

Table 3.

Mobile phase conditions

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	---	2.0	100.0	0.0	---
2	30.0	2.0	0.0	100.0	7
3	31.0	2.0	100.0	0.0	6
4	35.0	2.0	100.0	0.0	6
5	36.0	2.00	100.0	0.0	6

The UV spectra of the analytes were measured with an Aligent UV-Vis spectrophotometer© with UV-Vis Chem Station© software. At concentrations of 10mg/L, the UV absorption maxima of catechin, epicatechin, and epigallocatechin gallate were determined to be 280nm; caffeine was 270nm. Therefore, chromatograms were collected using analytical wavelengths of 270 and 280nm.

Peak detection and verification

The detection system was set to collect data at four wavelengths: 260nm, 270nm, 280nm, and 290nm. These wavelengths were used for peak verification, however only peaks from the 270nm and 280nm chromatograms were used for peak ratio analysis and chemical quantification.

Data analysis

Each standard was transferred into an autosampler vial and analyzed in the HPLC for 36 minutes to find each standard‘s unique retention time. The two mobile phases, acetonitrile/ 0.25% acetic acid (40%/60%, v/v) running at 2.0mL/min and 0.25% acetic acid running at 0.0mL/min. Each tea sample was injected and processed through the HPLC for 36 minutes in the same manner as each of the analyte standards. Detected and recorded wavelengths for all solutions were 260nm, 270nm, 280nm, 290nm; close to λmax of the analytes under investigation.

Computer generated HPLC standard solution chromatograms illustrated a solution‘s polyphenol and methylxanthine compounds. Standard solution peak areas from which the concentrations of corresponding polyphenols and methylxanthines extracted from the Lipton© White Tea were calculated using graphical quantification produced by the computer program, Graphical Analysis by Vernier©. Mean concentrations and their standard deviations were determined using Microsoft Excel. Statistical analysis was performed using *t*-test via the College of Saint Benedict and Saint John‘s University (SPSS).

Limitations of the study

The white tea extraction process of the analytes is a limit of the study. The quantification of polyphenols and the methylxanthine, caffeine by HPLC is dependent on the proficiency of the extraction process. The HPLC instrumentation is another limitation in that flavanol and

methylxanthine concentrations are based on the HPLC machine‘s ability to accurately quantify the components under analysis.

Chapter IV: Results and Discussion

Chapter four includes results obtained and a discussion related to the research findings. In review, Lipton® White Tea with Blueberry and Pomegranate flavoring was brewed under predetermined time intervals (2, 5, 10 minutes, and 24 hours) and temperatures (80°C, 85°C, 90°C, 95°C, and 100°C) to investigate its polyphenols (catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG)) and the methylxanthine (caffeine (Caf)) content. Each of the triplicate samples was then tested with reverse-phase HPLC methodology (Kaspar, 2006) in order to quantify polyphenol and methylxanthine concentrations in the white tea.

Tested component standard, tea polyphenol, and caffeine peak areas were given via HPLC chromatogram and graphed using a program called Graphical Analysis (Beaverton, Oregon). The specific tea components under investigation have varied maximum absorptions; therefore, multiple wavelength detections (260nm, 270nm, 280nm, and 290nm) were used. Preferred wavelengths were identified as they allowed for greater absorption and peak area in each constituent‘s concentration. Figure 6 shows a chromatogram of standard solutions used to determine the retention time of each of the analytes. The earliest analyte retention time was that of catechin at 17.9 minutes, second was caffeine at 18.5 minutes, third was epicatechin at 20.3, and finally EGCG at 20.6 minutes. The peak area of each analyte indicates its concentration in a given solution. The peak area varies under differing wavelengths.

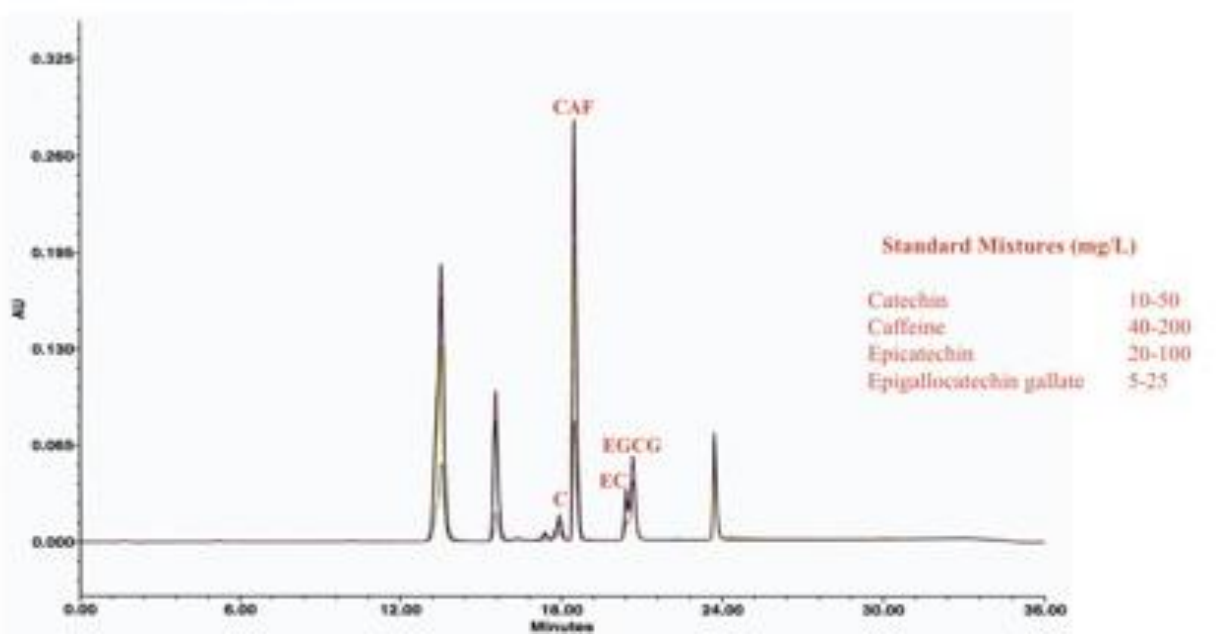

Figure 6. HPLC chromatogram of standard chemical retention times (Kafley, 2008)

Peak areas were greatest under wavelengths measuring 280nm/ 270nm in each tea chromatogram. Analyte peak ratios were compared to corresponding standard peak ratios with similar retention times. Each polyphenolic and methylxanthine concentration was determined using the equation formed by the best-fit line of the standard linear curve of each component.

Figure 7 shows the standard linear curve obtained from the concentrations of standard and the peak areas at varied wavelengths. The standard curve was used to calculate the amount of each component of polyphenols present in each tea sample under analysis.

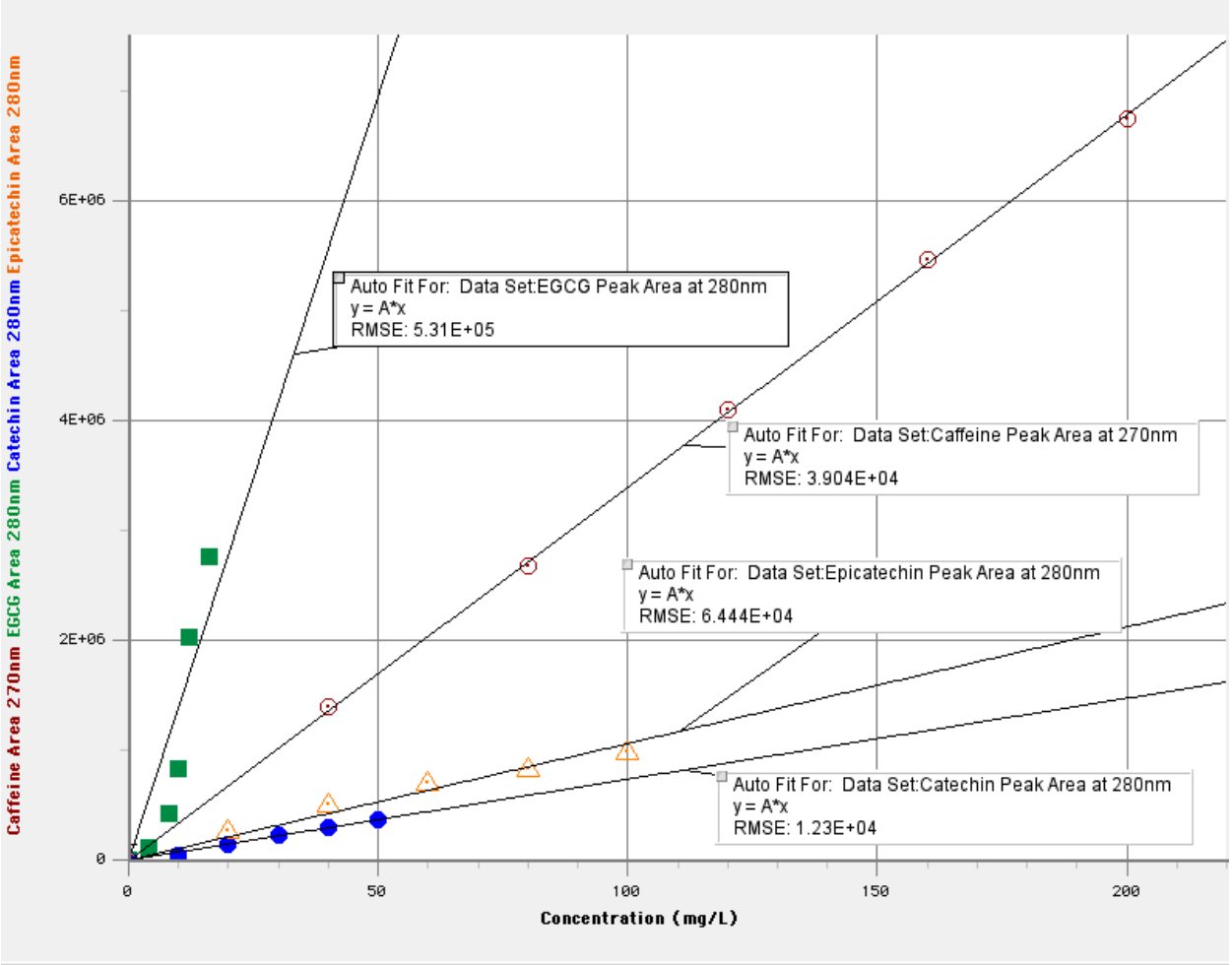

Figure 7. Curves of known standard component concentrations per HPLC analysis

The amounts of polyphenol and caffeine expressed in the HPLC analysis were analyzed and the results are shown in Table 4. Catechin concentrations generally increased as steeping duration and temperature increased. Starting at 8mg/250mL at 85, 90, 95, and 100°C to 22mg/250mL. In 80 and 85°C samples, catechin concentrations decreased between the 10-minute and 24-hour time increments. Epicatechin also increased in concentration from 2-minute to 24-hour steeping temperatures, with the exception to the 80°C samples. In the 80°C samples, epicatechin concentrations increased from 4.8mg/250mL at 2 minutes to 35.8mg/250mL at 10 minutes steeping time. The concentrations then decreased to 28.8mg/250mL at 24 hours of

steeping time. The greatest epicatechin concentration was 36.1mg/250mL at 100°C for 24 hours steeping duration. This suggests that the steeping temperature, which will express the largest concentration of epicatechin within 24 hours of steeping, is 100°C. The component EGCG increased in concentration initially for the temperature of 100°C from 2 minutes (12.5mg/250mL) to 24 hours (30.4mg/250mL). In tea samples brewing in temperatures at or below 95°C, EGCG levels decreased to at or below initial 2-minute steeping duration levels, with the exception of EGCG concentrations observed in 24-hour samples brewed at an initial temperature of 85°C. The largest EGCG concentration was observed at 100°C after a 24-hour steeping duration. This suggests that EGCG is expressed from tea at high temperatures (greater than 95°C) and steeping temperatures of at least 10 minutes.

Table 4

Analyzed polyphenol and methylxanthine concentrations (mg/250mL serving)

Brew Temperature	Brew Times	Catechin	Epicatechin	EGCG	Total Polyphenols	Total Caffeine
80°C	2 Minutes	9.00±2.0	10.2±1.9	4.80±0.8	24.0	62.0±2.6
	5 Minutes	11.0±1.0	25.8±1.6	6.40±0.1	43.2	84.5±2.6
	10 Minutes	13.0±2.0	35.8±1.9	5.40±1.4	54.2	85.0±3.0
	24 Hours	10.0±1.0	28.8±1.5	3.00±0.1	41.8	125±2.0
85°C	2 Minutes	8.00±1.0	9.90±1.7	3.50±0.2	21.4	64.3±1.3
	5 Minutes	11.0±3.0	15.0±0.1	9.10±0.2	35.1	101±1.5
	10 Minutes	15.0±2.0	20.1±1.9	15.3±0.6	50.4	125±2.2
	24 Hours	13.0±2.0	27.8±2.0	4.50±1.4	45.3	136±1.4
90°C	2 Minutes	8.00±1.0	15.1±0.1	9.30±1.9	32.4	86.7±3.0

	5 Minutes	9.00±1.0	17.6±0.2	11.5±0.3	38.1	105±1.7
	10 Minutes	12.0±1.0	20.5±0.3	15.3±0.2	47.8	122±1.8
	24 Hours	16.0±1.0	35.5±1.9	9.60±1.3	61.1	140±1.1
95°C	2 Minutes	8.00±1.0	14.1±0.3	9.90±0.1	32.0	74.3±2.0
	5 Minutes	12.0±1.0	20.8±1.9	17.4±1.0	50.2	113±0.5
	10 Minutes	13.0±3.0	21.2±1.9	20.5±2.2	54.7	122±1.8
	24 Hours	18.0±2.0	34.6±2.0	9.30±0.8	61.9	136±4.0
100°C	2 Minutes	8.00±2.0	16.3±0.1	12.5±0.1	36.8	94.3±2.2
	5 Minutes	8.00±1.0	21.4±1.9	17.5±1.9	46.9	116±1.1
	10 Minutes	11.0±1.0	24.0±2.0	20.8±1.9	55.8	145±2.1
	24 Hours	22.0±2.0	36.1±1.9	30.4±0.8	88.5	167±1.6

In Table 4, 2-minute samples for all temperatures, tended to increase in EGCG concentrations as steeping temperatures increased while the greatest concentration was expressed in 100°C steeping temperature (12.5mg/250mL); 5-minute samples also increased in EGCG concentrations up to 95°C and did not differ significantly from the sample steeped for 5 minutes at 100°C (17.4 and 17.5mg/250mL, respectively). The samples steeped for 10 minutes generally increased in catechin, epicatechin, and EGCG concentrations from the 2- and 5-minute samples at corresponding temperatures with the exception of EGCG at 80°C (Table 4). Concentrations at 100°C were greater ($P<0.05$) at 24 hours steeping than when brewed at 80°C for 24 hours brewed for other time intervals identified in Table 4 and Figure 8. This shows that larger concentrations of EGCG are expressed when steeped at 100°C rather than 80°C (Table 4). In Table 4, it is noted that in 24-hour samples steeped at all temperatures below 100°C were lowest

in concentration (less than 10mg/250mL). This may indicate that EGCG could decline over time, in low steeping temperatures, since EGCG concentrations are reduced between the 10-minute and 24-hour steeping times in all conditions except for 100°C. EGCG concentrations in samples steeped at 95°C and 100°C show similar concentrations at 5- and 10-minute steeping conditions. This suggests that brewing tea at five and ten minutes at temperatures of 95°C and greater would deliver the same health benefits of EGCG among the two different steeping temperatures.

In a previous study on green teas by Gudala (2008), Salada®, Bigelow®, and Celestial Seasonings® (caffeinated and decaffeinated) teas were studied to find and compare polyphenol and methylxanthine concentrations among these brands. Under extraction environments similar to those outlined in this paper, the teas expressed the greatest average concentrations of C, EC, and EGCG in Salada® Caffeinated Green Tea. Total polyphenol concentration generally increased with steeping times of 2-6 minutes, then declined at 10 minutes. The largest amount of total polyphenols (including theobromine, catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate) was observed at a temperature of 100°C with an 8-minute steeping time (239mg/250mL serving). The greatest catechin concentration (48.4±1.8 mg/250mL serving) was found when Salada® tea was steeped for 8 minutes at 80°C. The greatest epicatechin concentration (23.3±10.5mg/250mL serving) was found when Salada® tea was steeped for 8 minutes at 100°C. The greatest epigallocatechin gallate concentration (124±2.0 mg/250mL serving) was found when Salada® tea was steeped for 10 minutes at 80°C. No specific EGCG concentration trend was observed.

In this current study, the environment which produced the greatest concentration of all polyphenols was with 100°C spring water at a single long-term steeping time of 24 hours in

contrast to Gudala's findings in which the steeping environment to produce the greatest total polyphenol concentration (including theobromine, catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate) was found in Salada® Caffeinated Green Tea at 80°C for 8 minutes (239mg/250mL serving). The amounts of polyphenols observed in Salada® Caffeinated Green Tea were generally greater than in Lipton® White Tea. Gudala showed a catechin concentration of 26.4 more milligrams per 250mL serving (120% greater C than was found in Lipton® White Tea), an epicatechin concentration of 12.8 less milligrams per 250mL serving (55% less EC than was found in Lipton® White Tea), and an epigallocatechin gallate of 93.6 more milligrams per 250mL serving (308% greater EGCG than was found in Lipton® White Tea). In comparing these findings, it can be inferred that Salada® Caffeinated Green Tea generally has a greater polyphenolic concentration than Lipton® White Tea with Blueberry and Pomegranate.

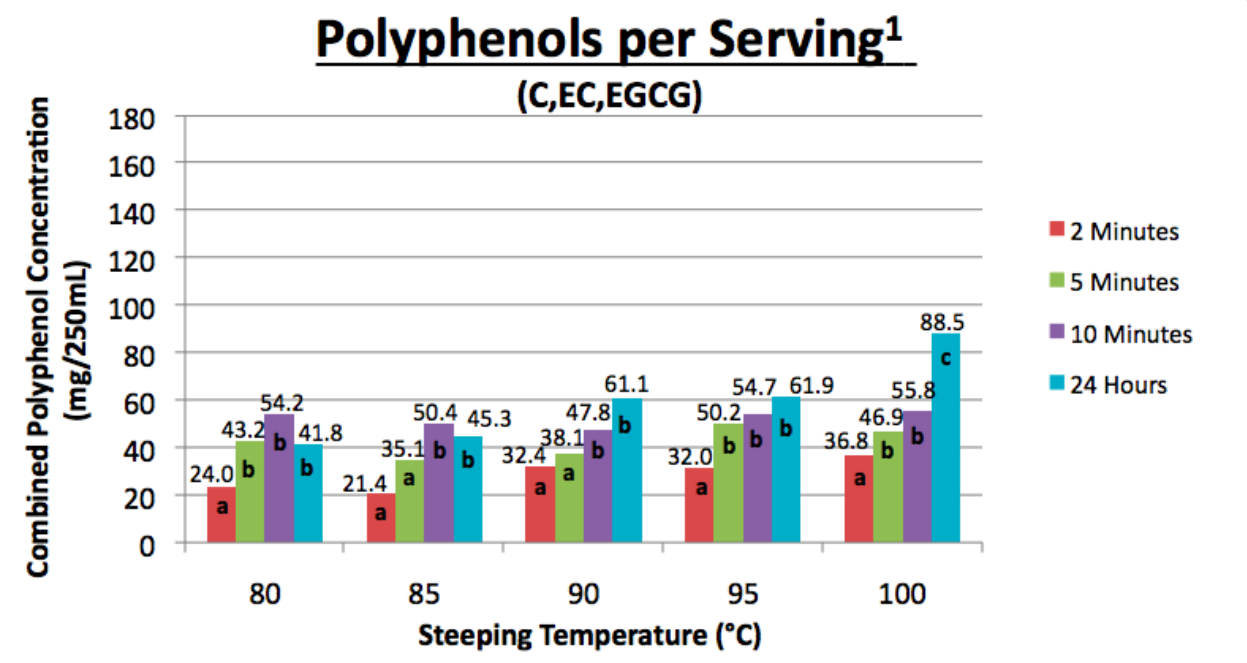

Figure 8. Total polyphenol concentration (mg/250mL serving) after steeping for 2, 5, 10 minutes, and 24 hours at several steeping temperatures

[1] Identified means (mg/250mL) within each temperature followed by different lowercase letters are significantly different ($P<0.05$)

Figure 8 illustrates the total polyphenolic concentrations that were compared to the corresponding peak ratios of known compounds with similar retention times. The concentration of each component in a sample was determined using the standard curve of each component. Findings generally show, that combined polyphenol concentrations increase with steeping temperature increases and duration. The overall total polyphenol content for each steeping condition ranged between 21.4mg/250mL to 88.5mg/250mL as shown in Table 4 and Figure 8, demonstrating that 100°C for a 24-hour steeping time allows the largest total polyphenols to be extracted from the white tea.

EGCG is a commonly investigated polyphenol due to its health benefits including cancer preventative properties (Lang et al., 2000). A study conducted by Jung and Ellis (2001) showed that EGCG is shown to inhibit tumor invasion and angiogenesis in cells. Table 5 shows that as the steeping temperatures increased, the greatest percentage of EGCG increased and was particularly large at 95°C and 100°C for 10 minutes (37%). This shows that steeping tea at 95°C or 100°C for 10 minutes results in the most optimal steeping condition in achieving a large concentration of EGCG. This finding is beneficial as EGCG is an important antioxidant for improving health.

Table 5

Content (mg/250mL, %/250mL) of EGCG among analyzed polyphenolic catechins

Steep Temp	Time Steeped	EGCG (mg/250mL)	Total Polyphenols (mg/250mL)	Percent EGCG per 250mL
80°C	2 Minutes	4.80±0.8	24.0	20%
	5 Minutes	6.40±0.1	43.2	15%
	10 Minutes	5.40±1.4	54.2	10%
	24 Hours	3.00±0.1	41.8	7%
85°C	2 Minutes	3.50±0.2	21.4	16%
	5 Minutes	9.10±0.2	35.1	26%
	10 Minutes	15.3±0.6	50.4	30%
	24 Hours	4.50±1.4	45.3	10%
90°C	2 Minutes	9.30±1.9	32.4	29%
	5 Minutes	11.5±0.3	38.1	30%
	10 Minutes	15.3±0.2	47.8	32%
	24 Hours	9.60±1.3	61.1	16%
95°C	2 Minutes	9.90±0.1	32.0	31%
	5 Minutes	17.4±1.0	50.2	35%
	10 Minutes	20.5±2.2	54.7	37%
	24 Hours	9.30±0.8	61.9	16%
100°C	2 Minutes	12.5±0.1	36.8	34%
	5 Minutes	17.5±1.9	46.9	37%

	10 Minutes	20.8±1.9	55.8	37%
	24 Hours	30.4±0.8	88.5	34%

Caffeine appears to be consistent in increasing in concentration as temperature and steeping durations increased, as Figure 9 shows that the caffeine in samples steeped for 2 minutes increased from the temperature of 80°C up to 100°C; caffeine concentrations in 5-minute samples steadily increased in caffeine concentration from 80°C to 100°C. The 10-minute steeping durations increased in caffeine concentrations from 80°C to 85°C, then decreased slightly at 90°C and again at 95°C, and increased to the greatest concentration at 100°C to 167.3mg/250mL serving (Figure 9). Caffeine concentrations were significantly greater ($p<0.001$) for 24-hour samples compared to all other times within each steeping temperature (Figure 9, Appendix A). This implies that the largest caffeine extraction for any steeping duration will be the most efficient in concentration at 100°C for 24 hours. All other steeping conditions were significant ($P<0.05$) at lesser confidence intervals between 2-minute and 5-minutes samples at all steeping temperatures; 5-minute and 10-minute samples at all steeping temperatures; 10-minute and 24-hour samples at all steeping temperatures, and 2-minute and 10-minute samples at all steeping temperatures (Figure 9), indicating that as steeping times increase, concentrations of caffeine increase.

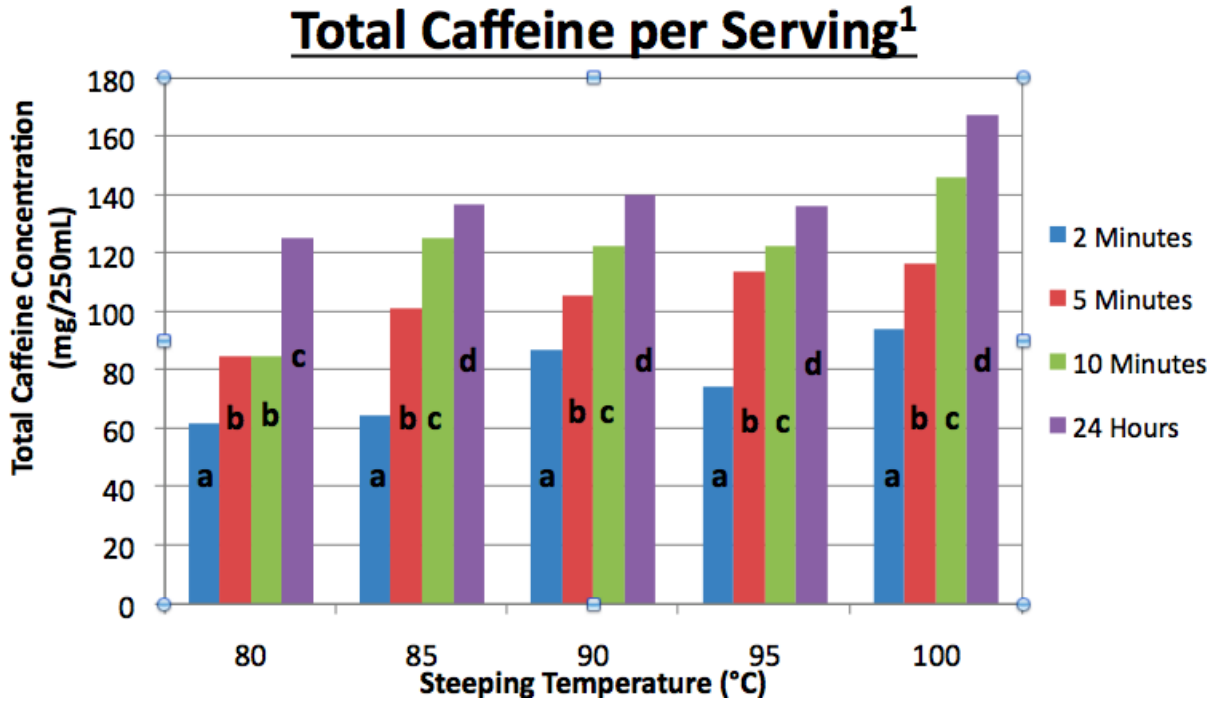

Figure 9. Total caffeine concentration (mg/250mL serving) after steeping for 2, 5, 10 minutes, and 24 hours at several temperatures

[1] Bars with different lowercase letters within each temperature are significantly different ($P<0.05$)

In Gudala's (2007) study, the greatest caffeine concentration (58.3±1.9 mg/250mL serving) was observed at 80°C when steeped for 10 minutes. This concentration is 108.7mg less per 250mL serving than that of the caffeine in Lipton® White Tea, which had 186% more caffeine per 250mL serving. This compared data shows that Lipton® White Tea has greater caffeine concentrations than Salada® Caffeinated Green Tea.

Chapter V: Conclusions

This study‘s purpose was to identify and quantify polyphenols and methylxanthines in Lipton® White Tea with Blueberry and Pomegranate flavoring at different steeping time and temperature conditions. Polyphenol and methylxanthine compounds were analyzed by HPLC. All of the concentrations were calculated in milligrams per serving consisting of 250mL spring water and 1 tea bag. All extraction conditions were tested in triplicate and findings were represented in average polyphenol and methylxanthine concentrations.

Generally, polyphenolic and caffeine concentrations increased directly as steeping times and temperatures increased. EGCG was unique in that after 10 minutes of steeping at all temperatures over 95°C, its concentration decreased. The decrease in concentrations after time may be related to the oxidation sensitivity of EGCG, which is higher than that of catechin or epicatechin after a longer exposure to room air and light (Teizer, 2005). This study‘s research shows that caffeine is a very stable chemical component that is expressed increasingly with heat and steeping duration. The findings in this study conclude that steeping conditions to extract the greatest concentration of EC, C, and caffeine are 24-hour steeping time with the initial steeping temperature of 100°C. Heating spring water to a full boil (100°C) when preparing Lipton® white tea with Blueberry and Pomegranate flavoring will extract the greatest antioxidants (EGCG, EC, and C) concentrations.

The findings of this research performed on white tea showed a similar trend to those recorded by Gudala (2008) on the research performed on various brands of commercially available green tea. Gudala‘s research showed a large concentrations of polyphenols and methylxanthines in Salada® green tea steeped for 10 minutes at 100°C initial temperature.

When comparing these results with those of a previous study (Gudala, 2008), the polyphenolic and methylxanthine concentrations in Salada® Caffeinated Green Tea had greater concentrations of catechin (120%), lower concentrations of epicatechin (55%), greater concentrations of EGCG (308%), and less concentrations of caffeine (186%) in previously identified steeping environments. This suggests that commercially available greens teas may have a greater catechin and EGCG concentrations than commercially available white teas.

From these findings and comparisons, both white and green teas provide polyphenols and methylxanthines that improve health in many areas as mentioned in chapter two. In order to obtain the greatest EGCG concentrations among these two tea brands/types, consumers may prepare tea brewed at 100°C for at least 10 minutes. Further efforts to elucidate more about polyphenols and methylxanthines in white tea and how to most completely express them in prepared tea samples is recommended.

Recommendations for further research include:

1. Conduct sensory analysis in testing preference between varied steeping times and temperatures to compare findings of sensory analysis to corresponding polyphenolic and methylxanthine concentrations.
2. Conduct sensory analysis in testing preference between varied tea types: black, oolong, green, white according to manufacturer preparation recommendations and relate the findings to known varied polyphenolic and/or methylxanthine concentrations.
3. Indentify and quantify polyphenol content of white tea at each hour up to 24 hours of discover at what point in time polyphenol concentrations decrease.
4. Identify and quantify polyphenol and methylxanthine concentrations in decaffeinated white tea.

5. Conduct similar extraction parameters on other brands of white tea to identify a difference among tea manufacturers.
6. Introduce vitamin C to brewed white tea to investigate antioxidative effects of vitamin C in efforts to preserve polyphenolic concentrations, including C, and EC, and EGCG.
7. Further compare polyphenolic and methylxanthine concentrations of C, EC, EGCG, and Caf in various commercially available white and green teas sold in the United States.

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Appendix A: p-Values in Caffeine Samples

	2 Minutes	5 Minutes	10 Minutes	24 hours
2 Minutes	---	0.003	0.003	0.000
5 Minutes	0.003	---	0.037	0.001
10 Minutes	0.003	0.037	---	0.015
24 Hours	0.000	0.001	0.015	---

Appendix A. Tested p-values showing significance in comparing time intervals in caffeine samples