Comparison of Antioxidant Loss During Storage of Freshly-Prepared and Ready-To-Drink Green Tea

Tomomi Sakata
Undergraduate Student, Senior Project in Food and Nutrition

Abstract

Green tea is an important source of antioxidants that can provide protection from cancer and other diseases. Unlike black and oolong, green tea processing does not involve a fermentation stage. Consequently, the antioxidant content of green tea is maintained. The antioxidants in green tea are polyphenols such as catechin, epicatechin, and related compounds. The most important antioxidant in green tea is epigallocatechin gallate (EGCG). Antioxidants are readily degraded by light, heat, and oxygen. Green tea popularity has resulted in a wide variety of commercial products such as bottled, flavored, deffafinated, and dehydrated teas. Bottled tea is called "ready-to-drink (RTD)," and the number of varieties of this convenient beverage is rapidly increasing. This study was carried out using high performance liquid chromatography (HPLC) to determine caffeine, EGCG, and ascorbic acid in both freshly-prepared herbal and ready-to-drink tea. Samples of either freshly-prepared or RTD products were opened and stored in a refrigerator. Caffeine proved to exhibit no loss during storage. However, ascorbic acid and EGCG were slowly degraded during storage.

Introduction

Green tea is one of most common beverages in the world (Shrubsole et al., 2008). Green tea has many health benefits including antioxidant activity and the prevention of cancer. Recently numerous green tea products, such as green tea extract chewing gum, toothpaste, shampoo, and body lotion, have been developed. In addition, many flavored green teas have become available. These include lemon, citrus, ginseng, mint, pomegranate, and mango flavor along with decaffeinated, and organic products. Traditional green tea is no longer the only form found in stores (Stauth, 2005). Now flavored tea and other tea products are found on shelves next to bottled beverages and soft drinks. The number of people who now consume or use green tea in one form or another is increasing. Both ready-to-drink (RTD) tea and tea bags are available in regular, flavored, and organic forms. Drinking green tea may reduce breast cancer, decrease the risk of dental caries, and prevent heart disease (Tea Association, 2009).

Green tea has catechins which are known to provide a health benefit due to their role as antioxidants. Catechins are polyphenols, and there are six important catechins found in tea: catechin (C), gallocatechin (GC), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG).

Green tea also contains the methylxanthine stimulants caffeine (Caf) and theobromine (Tb). Green tea may contain added ascorbic acid (AA). Ascorbic acid is commonly known as vitamin C. This study is a comparison of the catechins, ascorbic acid, and caffeine in bottled tea and herbal fresh tea (Yamamoto et al., 1997).

Materials and Methods

Reagents

Ascorbic acid, caffeine, catechin, epicatechin, gallic acid, and epigallocatechin were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO. HPLC-grade acetonitrile and reagent-grade 99% acetic acid were purchased from Fisher Scientific, Fairlawn, New Jersey.

HPLC required a solvent gradient. Solvent A (99.5%

water/0.25% acetic acid) was prepared by diluting 5 mL reagent-grade acetic acid to the mark in a 2-L volumetric flask with Milli-Q water. Solvent B was prepared by measuring 400 mL acetonitrile in a 1000-mL graduated cylinder and diluting to 1000 mL with solvent A.

Stock and Standard Solutions

Caffeine, 1000 mg/L. 1000 mg of the anhydrous solid was diluted to 1.000 L with 50:50 (v/v) methanol-Milli-Q water using a 1000-mL volumetric flask.

Catechin and epicatechin, 1000 mg/L. 100 mg of the anhydrous solids were diluted to 100-mL in separate volumetric flasks with Milli-Q water.

Standard solutions. Standards were prepared by pipetting the stock solutions and by direct weighing of solid AA and EGCG into 25-mL volumetric flasks followed by diluting to the final volume with HPLC-grade Milli-Q water.

Standard Mixtures

Standard mixtures of caffeine, ascorbic acid, catechin, gallic acid, epicatechin, and epigallocatechin, were prepared by adding appropriate volumes of stock solutions to 100-mL volumetric flasks and diluting to the mark with Milli-Q water. Due to the high expense of the polyphenol EGCG, milligram quantities were measured into 25-mL volumetric flasks in amounts appropriate to the desired concentrations. Similarly, milligram quantities of AA were directly measured into the 25-mL volumetric flasks due to the shelf life and instability of ascorbic acid stock solutions. Standard mixtures were used to fill these flasks to the 25-mL mark. By this technique, the concentration of caffeine, catechin, and epicatechin remained unchanged, and the concentration of AA and EGCG could be calculated from the dilution. A chromatogram of a standard mixture is shown in Figure 1 and standard curves for the polyphenols are shown in Figure 2 (Ondrus, 2008).

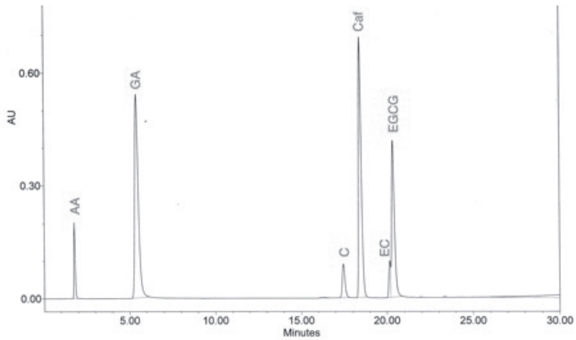

Figure 1: Chromatogram of a Standard Mixture Using 270-nm Detection

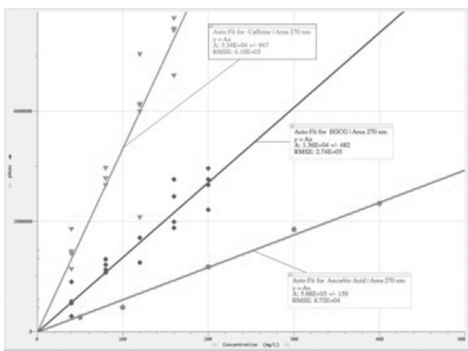

Figure 2: Standard curve of ascorbic acid, caffeine, and EGCG

Instrumentation

The Waters HPLC system used for this study consisted of an autosampler, binary pump, diode array detector, NovaPak C_{18} radial compression column, and Millenium chromatography software. Two wavelengths (270, and 280 nm) were used for detection and peak verification, but only the results from the 270-nm chromatograms are reported here because that wavelength is nearest the l_{max} for the analytes investigated.

Two aqueous solutions were used to generate a solvent gradient. Solution A consisted of 99.5% Milli-Q water/0.5% acetic acid (v/v). Solution B consisted of 40% acetonitrile and 60% solution A (v/v). Solvent composition was changed from 100% A to 100% B over a 30-minute run time. The gradient was slightly concave (nonlinear), corresponding to curve 8 on the Waters solvent programmer. 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.

Green Tea Preparation

The procedure for fresh green tea consisted of bringing spring water to a boil, transferring a 250-mL aliquot of the hot water to a 250-mL beaker, and steeping one tea bag in the beaker of hot water for 3.0 minutes. After removing the tea bag from the beaker, a portion was transferred to an autosampler vial with filtration through a 0.45 μm syringe-mounted membrane filter. The procedure for ready-to-drink teas was to transfer an aliquot to an autosampler vial with filtration through a 0.45 μm syringe-mounted membrane filter.

Results

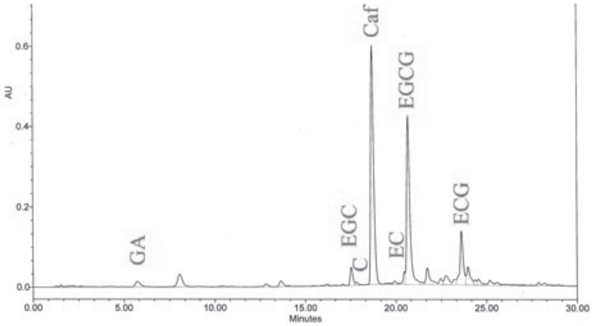

Figure 3: Chromatogram of Salada Green Tea Using 270-nm Detection

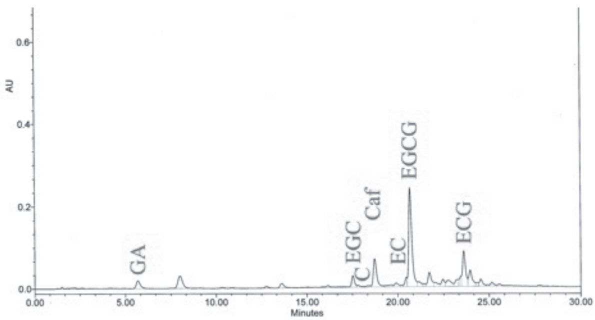

Figure 4: Chromatogram of Decaffeinated Salada Green Tea Using 270-nm Detection

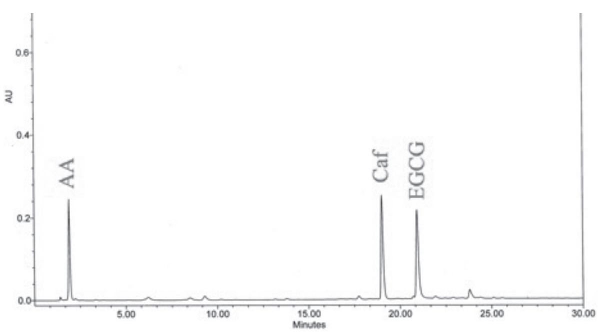

Figure 5: Chromatogram of Sobe Bottled Green Tea Using 270-nm Detection

Figure 3, 4, and 5 are examples of chromatograms of green tea samples. Figures 3 and 4 are chromatograms of Salada green tea and decaffeinated Salada green tea, respectively. The decreased caffeine peak size is apparent when comparing Figure 4 to Figure 3. Regular green tea tended to have higher peaks for most compounds including EGC, Caf, EC, and EGCG. Figure 5 is a chromatogram of Sobe Bottled Green Tea. This sample had three important peaks AA, Caf, and EGCG. The presence of ascorbic acid in the RTD product is apparent when comparing Figure 5 to Figures 3 and 4. Bottled tea also did not have many other antioxidant peaks when compared to fresh tea samples. Regular fresh green tea had the highest EGCG and caffeine peaks compared to the other two samples.

Table 1

Ascorbic acid, caffeine, and EGCG in ready-to-drink green tea

	Ascorbic Acid				Caffeine			
	Initial	After Day 28	Loss	% of Loss	Initial	After Day 28	Loss	% of Loss
Snapple Green	57	48.8	8.2	0.1	14.7	15.3	-0.6	0
Arizona	21.6	18.8	2.8	0.1	10.5	11.1	0.9	0.1
Itoen	43.2	37.4	5.7	0.1	25.2	25.3	-0.2	0
Lipton	43.5	42.5	1	0	12.8	13.2	1	0.1
Sench Shot	72.5	63.4	9.1	0.1	57	57.9	-0.9	0
Average	47.5	42.2	5.3	0.1	0	0	0	0

	EGCG			
	Initial	After Day 28	Loss	% of Loss
Snapple Green	36.1	35.9	0.2	0
Arizona	11.9	11.6	0.2	0
Itoen	14.9	14.5	0.4	0
Lipton	18.9	18.5	0.4	0
Sench Shot	44.7	34.8	9.9	0.2
Average	25.3	23.1	2.2	0.1

Table 2

Ascorbic Acid, Caffeine, and EGCG in herbal fresh green tea

	Ascorbic Acid				Caffeine			
	Initial	After Day 28	Loss	% of Loss	Initial	After Day 28	Loss	% of Loss
Lipton	ND	ND	ND	ND	37.5	36.3	1.3	3.3
Lipton Lemon Ginseng	ND	ND	ND	ND	24	23.5	0.5	2.1
Celestial Tropical Grape Fruit	ND	ND	ND	ND	24	24.5	-0.5	-2.1
Celestial Cranberry Pomegranate	ND	ND	ND	ND	26	26.3	-0.3	-1
HAIKU	ND	ND	ND	ND	32.5	32.3	0.3	0.8
Average	ND	ND	ND	ND	28.8	28.6	0.3	0.9

	EGCG			
	Initial	After Day 28	Loss	% of Loss
Lipton	56.3	11.3	45	80
Lipton Lemon Ginseng	28	8.3	20	70.5
Celestial Tropical Grape Fruit	14	8	6	42.9
Celestial Cranberry Pomegranate	28	1.8	26	93.8
HAIKU	38.3	10.8	28	71.9
Average	32.9	8	25	71.8

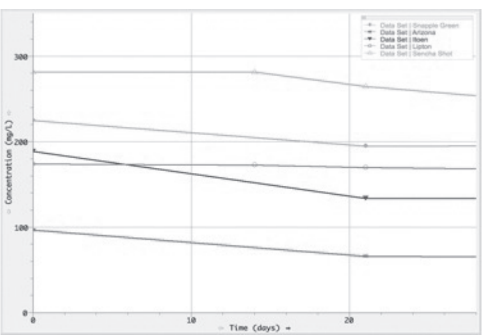

Figure 6: Graph of EGCG in bottled tea during 28 days.

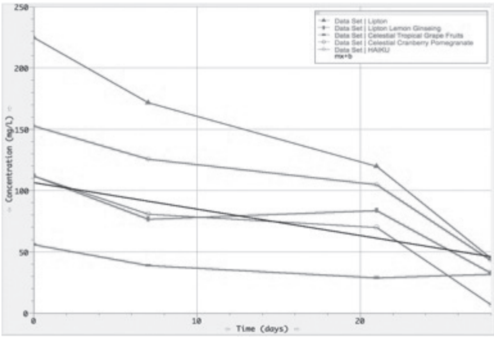

Figure 7: Graph of EGCG in herbal fresh tea during 28 days.

Tables 1 and 2 show the loss of EGCG, caffeine, and ascorbic acid by both ready-to-drink and freshly-prepared green tea during 28 days of storage in a refrigerator. Ascorbic acid was not detected in fresh herbal tea samples. The average loss by caffeine in fresh herbal tea was 0.9 % and bottled tea was 0.0 %. The average loss of EGCG in fresh herbal tea was 71.8 % whereas bottled tea was 0.1 %.

Comparing the ascorbic acid in both ready-to-drink and freshly-prepared herbal tea, bottled tea had ascorbic acid and lost a small amount after 28 days. Freshly-prepared tea did not contain a detectable amount of ascorbic acid. Both the ready-to drink and freshly-prepared herbal teas contained comparable caffeine levels and both demonstrated little tendency to decrease or increase. Freshly-prepared herbal tea initially had a higher EGCG concentration but it lost a larger amount after 28 days than bottled tea.

Figure 6 and 7 are plots of EGCG as a function of time for both ready-to-drink and freshly-prepared tea. The general trend is that the quantity of EGCG decreases with time. However, Figure 4 shows a greater slope indicating that freshly-prepared tea tends to lose antioxidants more rapidly than bottled tea.

Tables 1 and 2 illustrate that ready-to-drink teas contained ascorbic acid and freshly-prepared teas did not have ascorbic acid. According to Center for Science in Public Interest Food Safety, soft drinks and ready-to-drink beverages have larger amounts of ascorbic acid because they may slow down oxidation since ascorbic acid is an antioxidant (Center for Science, 2009). This research indicates that ready-to-drink green teas have a longer shelf life than freshly-prepared products. Ready-to-drink products contained smaller amounts of EGCG but it was more stable than fresh herbal tea for nearly 90 days with no observed mold. Table 2 shows that freshly-prepared herbal teas have a larger amount of EGCG initially than ready-to-drink products. According to the USDA Flavonoid Content database, freshly-prepared herbal tea has a larger amount of antioxidants and may provide more health benefits than ready-to-drink tea per serving. In all products, caffeine was a very stable compound.

Freshly-prepared green tea did not have any ascorbic acid, and ascorbic acid may be added during the process of bottling or canning tea. Bottled and canned green tea may have food additives for preservation in addition to ascorbic acid. Preservatives can make shelf life longer resulting in bottled and canned samples not having any mold after long storage when compared to fresh tea samples.

Discussion

Green tea products have been developed for a variety of consumer products. Green tea is well known as a healthy beverage which has antioxidants. Green tea has a health benefit to support a healthy life. Bottled tea was developed because it is convenient to drink green tea for more people.

According to the Lipton green tea ingredient list, the product contains 100% green tea. However, Diet Lipton ready-to-drink green tea with Citrus ingredients contains water, green tea, natural flavor, and some food additives.

The food additives, sodium hexametaphospate, calcium disodium EDTA, and ascorbic acid, are used to protect flavor and antioxidant activity. Phosphoric acid, potassium sorbate, and potassium benzoate preserve freshness by maintaining constant pH and help prevent the loss of essential nutrients. Asparatame and acesulfame potassium are used for non-calorie sweetener. Caramel color, yellow 5, and blue 1 provide desired color.

The innovation of bottled tea allowed for convenient consumption of green tea. However, some bottled teas, such as TAZO Organic Iced Green tea and Arizona Pomegranate Green Tea, have 70 calories for each 8 fl oz (240-mL) serving because sugar is used as the sweetener. Some bottled green teas are low in calorie content because aspartame is used as a sweetener. Freshly-prepared tea has no calorie content, and consumers may manage the sweetness by adding sugar or artificial sweeteners.

According to Low, Chin, Deurenberg-Yap, (2009), the United States has the highest percentage of its population that is overweight or obese. This can cause diabetes, cardiovascular disease, stroke, and some cancers. Since 1970, American portion size has grown and access to extra calorie intake has become increasingly easy (Young, & Nestle, 2002).

This study illustrated that ready-to-drink green tea beverages contain significant levels of ascorbic acid and the antioxidant EGCG. In fact, RTD products contain higher levels of ascorbic acid than freshly-prepared teas. It also verified that these RTD products retain antioxidant activity during storage better than freshly-prepared green tea. However, higher levels of the important antioxidant EGCG and fewer calories are found in green tea that is freshly-prepared from tea leaves (tea bags) by the consumer.

References

An Overview of Research on the Potential Health Benefits of Tea, Retrieved April 5, 2009, from Tea Association of the USA http://www.teausa.org/general/teaandhealth/218g.cfm

Food Additives, Retrieved April 4, 2009, from Center for Science in Public Interest, Food Safety http://www.cspinet.org/reports/chemcusine.htm

Low, S., Chin, Mien, & Deurenberg-Yap, M. (2009). Review on epidemic of obesity, Annals

Academy of Medicine Vol. 38 No.1

Ondrus, M. G., (2008). Caffeine, Theobromine, and Polyphenols in Green Tea, UW-Stout CHEM-335/535, Instrumental Methods student generated data.

Shrubsole, J. M., Lu, W., Chen, Z., Shu, O., Zheng, Y., Dai, Q., Cai, Q., Gu, K., & Zheng, W., (2009). Drinking Green Tea modestly reduces breast cancer risk. *The Journal of Nutrition*, 139(2), 310-316

Stauth, D., (2005) *HEALTH ISSUES UNCERTAIN AS TEA SALES BOOM,* Retrieved April 4, 2009, from OSU News & Communication Services http://oregonstate.edu/dept/ncs/newsarch/2005/Oct05/teaprotection.htm

USDA Database for the Flavonoid Content of Selected Foods Release 2.1 Prepared by the Nutrient Data Laboratory Food Composition Laboratory Beltsville Human Nutrition

Research Center Agricultural Research Service U.S. Department of Agriculture 87-88

Yamamoto, T., Juneja, J., Chu, D., & Kim, M. (1997) Chemistry and Applications of Green Tea.

Boca Raton, Frolida: CRC Press LLC

Young, L., & Nestle, M., (2002). The Contribution of Expanding Partion Sizes to the US obesity epidemic, American Journal of Public Health Vol. 92. No2 246-249