ANALYSIS OF THE ANTIOXIDANTS IN HOMEMADE VS. COMMERCIAL KOMBUCHA

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Abstract

Kombucha is tea that is fermented by a symbiotic culture of bacteria and yeast (SCOBY). It has many purported health benefits, partly due to its antioxidant levels. This study contrasted the concentrations of antioxidants in a variety of homemade vs. commercial kombucha teas. High performance liquid chromatography (HPLC-UV) was used to determine individual concentrations of caffeine, catechin, epicatechin, and epigallocatechin gallate (EGCG). UV-Vis spectroscopy was used to determine the concentrations of total antioxidants in the kombucha samples. The concentrations of caffeine, catechin, epicatechin, EGCG, and total antioxidants were all shown to be statistically higher in homemade kombucha than in commercial kombucha ($p < 5\%$). These differences were found to be independent of the fermentation process and of the specific SCOBY used, and they indicate that homemade kombucha is significantly higher in antioxidants than commercial kombucha.

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Introduction

Kombucha is tea that has been fermented by a symbiotic culture of bacteria and yeast (SCOBY). The starter tea typically contains black tea (*Camellia sinensis*)[1]. Upon the addition of table sugar and the SCOBY to the starter tea, the ethanol fermenters in the bacteria and yeast begin to metabolize sucrose by cleaving the glycosidic bond between glucose and fructose[1,2]. The formed monosaccharides then enter glycolysis, and ethanol is produced as a by-product. The acetic acid fermenters in the SCOBY then convert ethanol into acetic and lactic acids. The kombucha fermentation takes place in two stages: aerobic and anaerobic. Aerobic fermentation occurs while the tea with the SCOBY is exposed to air for a period of time, then anaerobic fermentation occurs after the SCOBY is removed and the tea is placed in airtight containers. Flavors such as berries or ginger are commonly added to the kombucha at the beginning of anaerobic fermentation. While the two stages of fermentation are common across kombucha brewing methods, the length of each stage can vary[1].

Kombucha has demonstrated health benefits, such as aiding with digestion, infections, stress, and even cancer, many of which are attributable to high levels of antioxidants[3,4]. Tea naturally contains antioxidants such as polyphenols, and the fermentation of kombucha reportedly produces more polyphenols[3-5]. These act as antioxidants because they neutralize free radicals and decrease the harmful accumulation of reactive oxygen species[6,7]. Flavonoids are one category of polyphenols in tea[7,8], and the flavonoids catechin, epicatechin, and epigallocatechin gallate (EGCG) are noted for their antimicrobial properties as well as antioxidant properties[9,10]. Caffeine also contains antioxidant properties and is inherently present in most teas[11].

Kombucha was originally made at home in small batches, but within approximately the last 20 years the commercial beverage industry began responding to increased demand for beverages which are healthier than typical soda, and kombucha started to become commercialized in the United States in the 1990s in an attempt to meet this demand[12]. However, it is questionable whether commercial and homemade kombuchas have equivalent antioxidant-causing health benefits. If commercial kombuchas contain fewer antioxidants, then customers seeking the benefits of drinking kombucha may not be justified in their consumption of mass-produced kombucha. The intention of this study was to determine whether homemade kombucha or commercial kombucha contains significantly more antioxidants and can therefore be considered healthier.

For this study, homemade kombucha was sampled after the home-brewer considered it to be “completed,” which occurred after varying fermentation lengths depending on the individual brewer. A general recipe is to boil 3-4 quarts of water for 10 minutes and then add 4-6 black tea bags and 1-cup of sugar and allow it to steep until the tea is at room temperature. After removing the tea leaves the beverage is poured into a sterilized jar and the SCOBY and kombucha starter (ca. 1/2 cup of previously made kombucha) are added. The jar is covered with a coffee filter or thin cloth and allowed to ferment for 7-14 days, after which the SCOBY is removed and the kombucha is strained and transferred into sealable containers where it ferments anaerobically and develops some carbonation over 1-2 weeks.[13] The kombucha is ready for consumption and can be saved (with refrigeration) for 2-3 months. Other kombucha recipes may vary in amount and type of tea (black is traditional), the amount of water and sugar, added flavors, the amount of fermentation time with the SCOBY, and the amount of time after bottling before being consumed. Samples were also obtained from various commercial kombuchas from local grocery stores. Caffeine, catechin, epicatechin, and EGCG in the samples were analyzed using high performance liquid chromatography with UV detection (HPLC-UV)[8,14]. Since fruit usually contains antioxidants, it was also anticipated that the kombucha with fruit additives would contain higher concentrations of antioxidants.

The samples were also analyzed using UV-Vis spectroscopy to determine the total concentration of antioxidants with intent to gauge the level of antioxidants not included in the four aforementioned polyphenols[10,15]. The Folin-Ciocalteau method was used with gallic acid as a standard. Gallic acid is commonly used as a standard in this type of analysis because it accurately represents the total quantity of phenolic antioxidants[15,16].

Materials and Methods

Antioxidant standards were obtained from Sigma-Aldrich, Inc.: (+)-catechin hydrate, (-)-epicatechin green tea, (-)-epigallocatechin gallate, caffeine. HPLC grade acetonitrile, acetic acid, and water were purchased from Fischer Scientific. Gallic acid and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich, Inc. A Dionex UltiMate 3000 pump and variable wavelength detector were used along with Chromeleon software (Chromeleon 7.2.10 ES, version MUa (24543)). The spectrometer used was an Agilent Technologies Cary 60 UV-Vis with CaryWinUV software (version 5.0.0.999).

Folin-Ciocalteu Phenol Reagent contains lithium sulfate, sodium tungstate, phosphoric acid, hydrochloric acid, and bromine. It is highly corrosive and dangerous to skin and eyes and should only be handled with personal protective equipment (gloves, lab coat, goggles) in a well-ventilated fume hood.

HPLC Analysis Method

All samples and standards were filtered through a 47 mm 0.45 μm nylon filter membrane. The mobile phase was comprised of acetonitrile and 0.1% aqueous acetic acid. A Phenomenex Gemini C18 5μm 4.6x150 mm column (20 μL sample loop) was used at a wavelength of 280 nm and a flow rate of 1 mL/min. A 30-minute gradient elution program began with 90% aqueous acetic acid (0.1%) and 10% acetonitrile and changed linearly to 84% and 16% over 10 minutes. It remained constant for 10 minutes, changed to 76% and 24% over 5 minutes, and then returned to the original 90% and 10% concentrations over the remaining 5 minutes[8].

Preparation of HPLC Standards

Caffeine, catechin, and EGCG standards were diluted with water to prepare 500, 400, 300, 200, and 100 ppm solutions, respectively. Epicatechin standard was likewise diluted to 500, 250, 100, and 50 ppm. Standard curves were calculated by plotting the known concentrations for each solution against their respective peak areas[8]. Each standard curve had a linearity reflected by a correlation (R^2) coefficient of at least 0.99.

Kombucha Analysis by HPLC

Homemade kombucha samples were obtained from six different local sources. Each source personalized their own kombucha, so differences between sources included the tea used, the SCOBYs, brewing methods, and flavors added. The homemade kombucha samples for this study were not chosen based on any type of criteria other than having been made locally within the previous two months. Since the intent was to show an overall statistical difference representative of kombucha in general, variation among the analyzed kombuchas was intentional. A wide variety of different flavors were also analyzed from a range of sources. The flavors were: plain (three different batches), blackberry-ginger, ginseng-turmeric, hibiscus-lime, strawberry-rhubarb-ginger, ango-ginger, cherry, lavender, strawberry, and pineapple.

A variety of commercial kombuchas were randomly selected for comparison and purchased from local groceries (Kroger, Walmart, Aldi). Six flavors were analyzed, each from a different brand. Duplicate bottles of each flavor were also purchased and analyzed for validity assurance purposes. Two of each of the following were analyzed: Simple Truth's Organic Blueberry Ginger, Bucha's Guava Mango, GT's Multi-Green, Brew Dr's Clear Mind, Vita Life's Ginger Awakening, and Health Ade's Pomegranate.

Each homemade and commercial sample was analyzed by HPLC-UV in triplicate, following the same method as the antioxidant standards. The samples were all filtered through a 0.45 micron nylon syringe filter (Whatman 7404-002) prior to injection. *HPLC concentrations (ppm) for each analyte for every homemade and commercial sample are presented in the supporting information.*

Preparation of Standards for Total Antioxidant Analysis

On each day of analysis, five gallic acid standards (200, 150, 100, 50, and 25 ppm) were prepared by diluting a 1000 ppm gallic acid stock solution with water. Deionized water (4.5 mL) and a sample of each of the gallic acid standards (0.5 mL) were individually added to six separate test tubes and vortexed. An aliquot of each diluted gallic acid (0.5 mL) was transferred to a secondary test tube. A blank was also prepared using DI water (0.5 mL) instead of diluted gallic acid. The following reagents were promptly added to each secondary test tube in order: deionized water (4.5 mL), Folin - Ciocalteu's reagent (0.2 mL), and saturated sodium carbonate (0.5 mL). After quickly vortexing each solution, additional deionized water (4.3 mL) was added to each test tube. Each tube was capped with a rubber stopper and inverted to mix. The standards remained at room temperature for a minimum of one hour and a maximum of three hours before analysis[15,16].

Kombucha Analysis for Total Antioxidants

Total antioxidant analyses were performed on the homemade kombucha flavors plain, ginseng turmeric, hibiscus lime, strawberry rhubarb ginger, mango ginger, cherry, lavender, strawberry, and pineapple. The commercial kombucha flavors were two of each of the following: Blueberry Ginger, Guava Mango, Multi-Green, Clear Mind, Ginger Awakening, and Pomegranate. An aliquot of each kombucha sample (0.5 mL) was diluted with deionized water (4.5 mL). This was repeated in triplicate, and a portion (0.5 mL) of each diluted sample was pipetted into a designated test tube and the same reagents and amounts were added as described above for the gallic acid standards (total volume 10.0 mL). Each tube was capped with a rubber stopper and inverted to mix. The samples remained at room temperature for 2-3 hours before analysis[15,16].

All standards and samples were analyzed in quartz cuvettes with a path length of 1 cm at a wavelength of 725 nm[15,16]. The different types of kombucha were analyzed on different days, but the gallic acid standards were re-made and analyzed each time. The concentration of gallic acid was used as a standard reference for the concentration of total antioxidants in the kombucha samples. The Cary WinUV software was used to calculate standard curves from the gallic acid standards every time new standards and samples were analyzed and was used to determine the concentration of total antioxidants in the kombucha samples. The concentration was multiplied by ten to account for the dilution of the sample. All standard curves had R^2 values >0.98. *Total antioxidants concentrations (ppm) for every homemade and commercial sample* *are presented in the supporting information.*

Statistical Analysis

The means were calculated for the concentrations of caffeine, catechin, epicatechin, EGCG, and total antioxidants in all the homemade and commercial samples. It was determined that all the assumptions for independent samples t-tests were met. The means for each antioxidant in the homemade samples were compared to the means for each antioxidant in the commercial samples using the independent samples t-test assuming unequal variance. Calculated experimental values for Student's t were compared to the literature values. If the p values calculated for the t-tests were below 0.05, or 5%, then statistical differences were indicated[17]. *The t-test calculations and results are available in the supporting information.*

Results and Discussion

Validation

A challenge to overcome for proper quantification of these specific analytes was the sample diversity. This heterogeneity of tea samples could potentially lead to matrix issues that could cause discrepancies in the measured antioxidants. To ensure that the kombucha matrix was not altering the measured antioxidant concentrations in homemade vs. commercial kombuchas, a sample of homemade (cherry) and of commercial (Clear Mind) kombucha was each spiked with 100 ppm of gallic acid. Each sample was analyzed in triplicate, and the areas of the gallic acid peaks were measured by HPLC-UV and compared to a gallic acid standard curve (25, 50, 100, 150, and 200 ppm). The average amount of gallic acid present in the homemade cherry kombucha was found to be108 ppm (108%) with a standard deviation of 3.77. The average amount of gallic acid in the Clear Mind commercial kombucha was found to be 110 ppm (110%) with a standard deviation of 3.42. Thus, there were no large differences or matrix issues found in the analysis of a known amount of gallic acid in homemade as compared to commercial kombucha. Since gallic acid is a phenolic antioxidant with similarities to the other phenolic antioxidants analyzed in this study, this validation indicates confidence in the reliability of the overall results and in the use of calibration curves made from the other antioxidants.

Analysis of Individual Antioxidants by HPLC-UV

The average concentrations in ppm (μg/mL) for each antioxidant in each homemade sample are shown in **Table 1**. On average, the antioxidants were present in the following order, from greatest to least: caffeine, EGCG, epicatechin, catechin.

The average concentrations in ppm (μg/mL) for each antioxidant in each commercial sample are shown in **Table 2**. On average, the antioxidants were present in the following order, from greatest to least: caffeine, EGCG, catechin, epicatechin.

Since the composition of kombucha can differ so greatly, a wide variety of kombucha types were intentionally chosen so that the overall results better represent kombucha in general. Kombucha is a craft process and reflects the individuality of the person making it. We obtained samples from six individuals who used varying amounts of tea, water, sugar and additives. This variability was intentional, as it mirrors the range of recipes utilized in commercial samples. The fluctuations in antioxidant profiles be-

Table 1: Antioxidant profile for homemade kombucha. The quantities of the four studied antioxidants are listed below for each of the homemade flavors. Dashes indicate where a particular antioxidant was not detected.

Flavor	Avg. ppm Caffeine	Avg. ppm Catechin	Avg. ppm Epicatechin	Avg. ppm EGCG
Plain, batch 1	81.9	11.6	9.8	36.4
Plain, batch 2	158.8	50.4	38.4	109.9
Plain, batch 3	94.9	15.3	18.8	39.1
Ginseng Turmeric	33.0	1.5	11.0	4.3
Hibiscus Lime	29.8	24.2	9.7	6.0
Strawberry Rhubarb Ginger	88.6	27.1	13.4	30.0
Mango Ginger	85.3	16.5	10.5	32.2
Cherry	75.4	2.1	9.6	51.5
Lavender	94.0	--	7.6	44.2
Strawberry	167.6	24.0	104.4	231.0
Pineapple	136.9	20.0	90.2	188.1
Total Average	93.0	19.0	30.1	70.6

Table 2 Antioxidant profile for commercial kombucha. The quantities of the four studied antioxidants are listed below for each of the commercial flavors. Dashes indicate where a particular antioxidant was not found.

Flavor	Avg. ppm Caffeine	Avg. ppm Catechin	Avg. ppm Epicatechin	Avg. ppm EGCG
Blueberry Ginger 1	32.1	14.4	1.0	3.6
Blueberry Ginger 2	31.8	14.2	2.7	3.7
Guava Mango 1	34.2	2.3	2.6	7.0
Guava Mango 2	35.1	5.6	3.2	7.5
Multi-Green 1	55.4	2.5	7.2	9.9
Multi-Green 2	57.6	--	6.2	7.2
Clear Mind 1	23.5	--	7.7	39.7
Clear Mind 2	17.7	--	5.7	44.2
Ginger Awakening 1	50.5	--	1.7	9.4
Ginger Awakening 2	53.5	--	1.6	4.9
Pomegranate 1	22.4	18.1	--	13.6
Pomegranate 2	43.5		--	25.5
Total Average	38.1	8.5	4.1	14.7

tween the different samples reflect the dramatic influence that factors such as brewing method, tea type, SCOBY type, and flavors can have on the beverage. The fact that the average antioxidants in homemade kombucha are so much greater than those in commercial kombucha, despite the variety of samples, suggests that there is a difference between home-brewed and industrial-brewed kombucha.

The most remarkable discrepancies between kombucha types lie in the average epicatechin and EGCG concentrations. The average epicatechin concentration was found to be about 7 times larger in homemade samples, and the average EGCG concentration was found to be about 5 times larger in the homemade samples than in the commercial samples. Additionally, the average caffeine and catechin concentrations are approximately twice as large in the homemade samples. These conclusions are shown to be significant based on t-tests which indicate statistical differences between the means of the four antioxidants in homemade vs. in commercial kombucha. The p values are all significantly below 0.05, indicating that the concentrations of these four antioxidants in homemade kombucha are indeed statistically different from those in commercial kombucha.

Additionally, t-tests were performed to compare the antioxidants in homemade kombucha containing fruit (7 samples) to the homemade kombucha without fruit (5 samples). The results for caffeine and catechin did not pass the t-test ($p>0.05$), but the results for epicatechin and EGCG did pass ($p<0.05$). This data is intriguing, and it indicates the need for further study on the addition of fruit to kombucha. It is possible that fruit-flavored kombucha might be the healthier type of kombucha from an antioxidant standpoint.

Analysis of Total Antioxidants by UV-Vis

The concentrations of total antioxidants in kombucha are shown for homemade kombucha in **Table 3** and for commercial kombucha in **Table 4**. Units are listed in parts per million of gallic acid equivalent.

T-test analysis shows that the p value comparing total antioxidants is significantly below 0.05 ($p = 6.128 \times 10^{-6}$), indicating that the concentrations of total antioxidants in homemade kombucha are statistically different from those in commercial kombucha.

Comparison of Total Antioxidants

The data in **Tables 3 and 4** show that homemade kombucha contains on average over twice the total antioxidants of commercial kombucha, just as the homemade samples contain more phenolic antioxidants. There may be multiple contributing reasons for these differences. It is possible that the industrial brewing methods for commercial kombucha dilute the tea more than small-batch brewers either for taste, economic, and/or other reasons. Also, the type of tea used to prepare the commercial kombuchas was not listed on any of the labels, and different teas have different antioxidant profiles. Additional factors that might affect antioxidant composition could be forced carbonation, fermentation length, or SCOBY microbiome profiles[4].

The overall conclusion of this study is that homemade and commercial kombuchas should not be considered equivalent with regards to the health benefits from antioxidants. While commercial kombucha does have appreciable amounts of antioxidants we found that homemade kombucha has much more. If kombucha is being consumed for a source of antioxidants, then the type of kombucha is clearly an important factor. There are many other differences between kombuchas, however, including microbiome composition, taste, sugar content, and pH. Some of these factors have

Table 3 Total antioxidants analysis in homemade kombucha

Flavor	Average Concentration Adjusted for Dilution in ppm
Plain Batch 1	559
Ginseng Turmeric	270
Hibiscus Lime	335
Strawberry Rhubarb Ginger	548
Mango Ginger	492
Cherry	605
Lavender	251
Strawberry	1078
Pineapple	942
Total Average	546

Table 4 Comparison between total antioxidants in commercial kombucha

Flavor	Average Concentration Adjusted for Dilution in ppm
Blueberry Ginger 1	266
Blueberry Ginger 2	234
Guava Mango 1	183
Guava Mango 2	245
Multi-Green 1	287
Multi-Green 2	259
Clear Mind 1	251
Clear Mind 2	360
Ginger Awakening 1	232
Ginger Awakening 2	220
Pomegranate 1	318
Pomegranate 2	319
Total Average	265

been researched[1,4,12] but further research uncovering impactful differences between home-brewed and industrial-brewed kombucha would be valuable. This study demonstrated for the first time, to our knowledge, that there are more antioxidants on average in homemade kombucha than are found in commercial kombucha[18].

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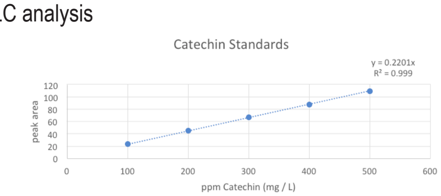
Supporting Information

I. Standard Curves for HPLC Analysis
   A) Catechin
   B) EGCG (epigallocatechin gallate)
   C) Epicatechin
   D) Caffeine
II. HPLC results and average antioxidant concentrations for each Kombucha
   a) Homemade kombuchas
   b) Commercial kombuchas
III. Total Antioxidant Analysis (ppm gallic acid equivalent) of Kombucha
   a) Homemade kombuchas
   b) Commercial kombuchas
IV. F and T-tests of HPLC Data (Homemade versus Commercial)
   a) F-tests
   b) T-tests
V. HPLC results and averages for selected Kombucha with and without fruit
   a) Fruit-containing kombucha
   b) No-fruit kombucha
   c) F-tests for Fruit vs. No-Fruit
   d) T-tests for Fruit vs. No-Fruit

I. Standard curves for HPLC analysis

A. Catechin Standard

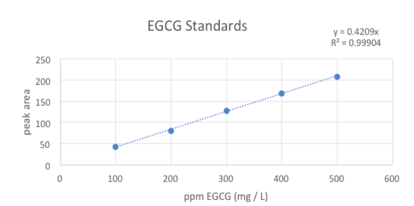
Catechin ppm	Peak Area
500	109.3709
400	87.5906
300	66.7174
200	44.6721
100	23.7528



A) Catechin

B. EGCG (Epigallocatechin gallate) Standard

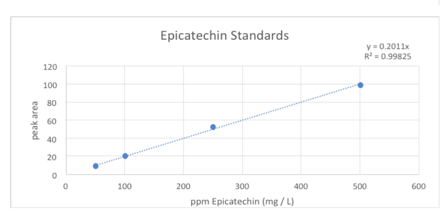
EGCG ppm	Peak Area
500	208.8844
400	169.3149
300	129.0326
200	81.8309
100	42.6936



B) EGCG

C. Epicatechin Standard

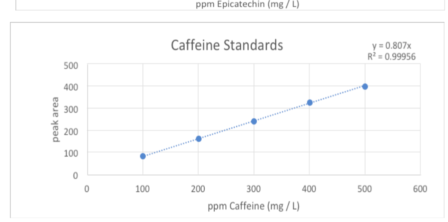
Epicatechin ppm	Peak Area
500	99.1959
250	52.6532
100	21.0558
50	9.9903



C) Epicatechin

D. Caffeine Standard

Caffeine ppm	Peak Area
500	399.9239
400	325.6765
300	242.8195
200	162.2159
100	83.1469



D) Caffeine

II. HPLC results and averages (ppm, mg/L) for each Kombucha

a) Homemade Kombucha HPLC Results (ppm)

Flavor	Caffeine	Avg Caffeine	Catechin	Avg Catechin	Epicatechin	Avg Epicatechin	EGCG	Avg EGCG
Plain Batch 1 A	85.351		15.27		12.221		36.418	
Plain Batch 1 B	77.249		8.285		11.276		36.335	
Plain Batch 1 C	83.084	81.895	11.238	11.598	5.938	9.811	34.178	36.377
Plain Batch 2	158.819	158.819	50.427	50.427	38.351	38.351	109.912	109.912
Plain Batch 3	94.914	94.914	15.306	15.306	18.793	18.793	39.051	39.051
Ginseng Turmeric 1	37.615		2.870		11.009		5.543	
Ginseng Turmeric 2	30.376		1.194		10.648		3.627	
Ginseng Turmeric 3	30.867	32.953	0.506	1.523	11.250	10.969	3.652	4.274
Hibiscus Lime 1	27.769		28.069		9.460		5.111	
Hibiscus Lime 2	33.883		22.093		10.722		6.912	
Hibiscus Lime 3	27.719	29.790	22.490	24.217	9.859	10.014	6.014	3.012
Blackberry Ginger	159.027	159.027	49.458	49.458	45.350	45.350	98.901	98.901
Strawberry Rhubarb Ginger 1	86.716		28.597		12.711		30.848	
Strawberry Rhubarb Ginger 2	89.534		27.042		13.483		31.230	
Strawberry Rhubarb Ginger 3	89.485	88.578	25.539	27.059	14.016	13.403	27.834	29.970
Mango Ginger 1	84.401		15.080		10.309		31.806	
Mango Ginger 2	84.727		16.046		10.311		32.535	
Mango Ginger 3	86.836	85.321	18.386	16.504	10.978	10.533	32.184	32.175
Cherry 1	76.085		1.968		8.974		56.118	
Cherry 2	75.385		2.074		9.597		48.201	
Cherry 3	74.745	75.396	2.201	2.081	10.178	9.583	50.186	51.501
Lavender 1	73.599		ND		6.650		35.614	
Lavender 2	104.934		ND		8.076		48.406	
Lavender 3	103.352	93.962	ND	ND	8.141	7.622	48.642	44.221
Strawberry 1	147.805		24.877		96.823		210.107	
Strawberry 2	204.233		26.685		115.879		259.569	
Strawberry 3	150.741	167.593	20.304	23.955	100.372	104.358	223.261	230.979
Pineapple 1	139.988		26.003		90.566		194.634	
Pineapple 2	135.389		16.148		90.115		185.796	
Pineapple 3	135.415	136.931	17.810	19.987	89.856	90.179	183.945	188.125
Total Avg.	93.001		18.369		30.064		70.552	

b) Commercial Kombucha HPLC Results (ppm)

Flavor	Caffeine	Avg Caffeine	Catechin	Avg Catechin	Epicatechin	Avg Epicatechin	EGCG	Avg EGCG
STO Blueberry Ginger 1	32.368		16.148		1.235		3.905	
STO Blueberry Ginger 1	31.788		12.116		0.853		3.452	
STO Blueberry Ginger 1	32.248	32.134	14.795	14.353	0.770	0.953	3.372	3.576
STO Blueberry Ginger 2	31.689		15.171		2.626		3.965	
STO Blueberry Ginger 2	31.314		12.221		2.599		3.362	
STO Blueberry Ginger 2	32.508	31.837	15.204	14.199	2.975	2.733	3.785	3.704
Bucha Guava mango 1	34.081		2.6344		2.677		6.679	
Bucha Guava mango 1	34.466		2.3498		2.660		3.893	
Bucha Guava mango 1	34.001	34.183	1.6136	2.199	2.557	2.632	7.283	6.952
Bucha Guava mango 2	35.434		1.788		2.021		8.729	
Bucha Guava mango 2	35.122		3.784		3.371		6.674	
Bucha Guava mango 2	34.726	35.094	1.195	2.255	4.149	3.180	7.139	7.514
GT Multi-green 1	54.732		6.6286		6.747		9.590	
GT Multi-green 1	55.471		5.0161		7.288		10.116	
GT Multi-green 1	55.951	55.385	5.2561	5.634	7.523	7.186	9.921	9.876
GT Multi-green 2	57.122		2.801		3.607		8.524	
GT Multi-green 2	58.476		3.079		5.681		7.120	
GT Multi-green 2	57.123	57.574	1.483	2.454	9.440	6.242	5.898	7.181
Dr. Brew Clear Mind 1	24.2345		ND		8.6158		40.8677	
Dr. Brew Clear Mind 1	24.0858		ND		7.8232		41.3827	
Dr. Brew Clear Mind 1	22.1001	23.4734	ND	ND	6.7428	7.727	36.9184	39.723
Dr. Brew Clear Mind 2	17.6776		ND		5.6022		35.6139	
Dr. Brew Clear Mind 2	17.7385		ND		6.0698		48.4063	
Dr. Brew Clear Mind 2	17.6946	17.7036	ND	ND	5.4525	5.708	48.6415	44.221
Vita Life Ginger Awakening 1	49.7605		ND		3.0462		6.8299	
Vita Life Ginger Awakening 1	50.8473		ND		1.2456		10.6986	
Vita Life Ginger Awakening 1	50.7638	50.4572	ND	ND	0.8388	1.710	10.6399	9.389
Vita Life Ginger Awakening 2	53.5269		ND		2.2441		4.4028	
Vita Life Ginger Awakening 2	53.4858		ND		0.9854		4.8849	
Vita Life Ginger Awakening 2	53.5029	53.5052	ND	ND	ND	1.615 (avg of 2)	5.3228	4.937
Health Ade Pomegranate 1	23.9681		ND		ND		14.9094	
Health Ade Pomegranate 1	21.1227		ND		ND		13.0590	
Health Ade Pomegranate 1	21.9772	22.3560	ND	ND	ND	ND	12.7317	13.567
Health Ade Pomegranate 2	42.9443		17.6892		ND		24.9574	
Health Ade Pomegranate 2	43.2422		17.2424		ND		24.4817	
Health Ade Pomegranate 2	44.2215	43.4693	19.3159	18.0825	ND	ND	27.1101	25.516
Total Avg.	38.098		8.454		4.050		14.68	

III. Total Antioxidant Analysis of Kombucha

a) Homemade Kombucha (Total Antioxidants Results)

[illegible]	Sample 1	[illegible]	[illegible]	[illegible]
	Sample 2	53.7	537	
	Sample 3	59.9	599	
Ginseng-turmeric	Sample 1	27.3	273	270
	Sample 2	26.4	264	
	Sample 3	27.3	273	
Hibiscous lime	Sample 1	33	330	335
	Sample 2	33.9	339	
	Sample 3	33.5	335	
Strawberry Rhubarb Ginger 1	Sample 1	52.7	527	548
	Sample 2	56.4	564	
	Sample 3	55.3	553	
Mango Ginger	Sample 1	50.1	501	492
	Sample 2	47.6	476	
	Sample 3	49.9	499	
Cherry 1	Sample 1	67.5	675	605
Cherry 2	Sample 2	54.4	544	
Cherry 3	Sample 3	59.7	597	
Lavendar 1	Sample 1	25.6	256	251
Lavendar 2	Sample 2	24.6	246	
Lavendar 3	Sample 3	25.2	252	
Strawberry 1	Sample 1	108.2	1082	1078
Strawberry 2	Sample 2	108.5	1085	
Strawberry 3	Sample 3	106.6	1066	
Pineapple 1	Sample 1	94.3	943	942
Pineapple 2	Sample 2	95.8	958	
Pineapple 3	Sample 3	92.5	925	
			Total average	564

b) Commercial Kombucha (Total Antioxidants Results)

Kombucha		Concentration (ppm)	Adjusted Conc. (ppm)	Avg Concentration (ppm)
STO blue ginger 1	Sample 1	28.9	289	266
	Sample 2	28.3	283	
	Sample 3	22.7	227	
STO blue ginger 2	Sample 1	21.2	212	234
	Sample 2	24.9	249	
	Sample 3	24	240	
Bucha Guava Mango 1	Sample 1	21.4	214	183
	Sample 2	16.2	162	
	Sample 3	17.3	173	
Bucha Guava mango 2	Sample 1	24.8	248	245
	Sample 2	24.1	241	
	Sample 3	24.7	247	
GT Multi-green 1	Sample 1	28.4	284	287
	Sample 2	29.3	293	
	Sample 3	28.3	283	
GT Multi-green 2	Sample 1	22.8	228	259
	Sample 2	26.2	262	
	Sample 3	28.8	288	
Dr. Brew Clear Mind 1	Sample 1	21.6	216	251
	Sample 2	26.3	263	
	Sample 3	27.4	274	
Dr. Brew Clear Mind 2	Sample 1	37.4	374	360
	Sample 2	35.7	357	
	Sample 3	34.8	348	
Vita Life Ginger Awakening 1	Sample 1	23.3	233	232
	Sample 2	23.6	236	
	Sample 3	22.8	228	
Vita Life Ginger Awakening 2	Sample 1	22.4	224	220
	Sample 2	21.7	217	
	Sample 3	22	220	
Health Ade Pomegranate 1	Sample 1	31.7	317	318
	Sample 2	32.3	323	
	Sample 3	31.3	313	
Health Ade Pomegranate 2	Sample 1	32.4	324	319
	Sample 2	31.9	319	
	Sample 3	31.4	314	
			Total average	265

IV. F and T-tests of HPLC Data (Homemade versus Commercia

a) F-tests, Homemade vs Commercial

Caffeine

F-Test Two-Sample for Variances

	Variable 1	*Variable 2*
Mean	93.0005293	38.0975491
Variance	2009.53463	178.102025
Observations	30	36
df	29	35
F	11.2830533	
P(F<=f) one-tail	1.1334E-10	
F Critical one-tail	1.79231431	

Catechin

F-Test Two-Sample for Variances

Mean		
Variance		
Observations		
df		
F		
P(F<=f) one-tail		
F Critical one-tail		

Epicatechin

F-Test Two-Sample for Variances

Mean		
Variance		
Observations		
df		
F		
P(F<=f) one-tail		
F Critical one-tail		

EGCG

F-Test Two-Sample for Variances

	Variable 1	*Variable 2*
Mean	70.5522631	14.6796209
Variance	5704.18741	190.655337
Observations	30	36
df	29	35
F	29.9188447	
P(F<=f) one-tail	2.8613E-17	
F Critical one-tail	1.79231431	

b) T-Tests (Homemade vs. Commercial)

Caffeine

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	93.0005293	38.0975491
Variance	2009.53463	178.102025
Observations	30	36
Hypothesized Mean Difference	0	
df	33	
t Stat	6.47344642	
P(T<=t) one-tail	1.2056E-07	
t Critical one-tail	1.69236031	
P(T<=t) two-tail	**2.411E-07**	
t Critical two-tail	2.0345153	

Catechin

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	18.3690462	8.4537988
Variance	167.967106	43.4932969
Observations	27	21
Hypothesized Mean Difference	0	
df	40	
t Stat	3.44326892	
P(T<=t) one-tail	0.00068063	
t Critical one-tail	1.68385101	
P(T<=t) two-tail	**1.361E-03**	
t Critical two-tail	2.02107539	

c) F-tests for Fruit vs. No-Fruit

Caffeine **Not pass**

F-Test Two-Sample for Variances

Caffeine

	Variable 1	*Variable 2*
Mean	100.518697	80.0146218
Variance	2277.00123	1436.15711
Observations	19	11
df	18	10
F	1.58548198	
P(F<=f) one-tail	0.23047835	
F Critical one-tail	2.79804506	

Catechin **Pass**

F-Test Two-Sample for Variances

	Variable 1	*Variable 2*
Mean	20.5720552	13.1369
Variance	123.412222	262.072508
Observations	19	8
df	18	7
F	0.47090869	
P(F<=f) one-tail	0.0938218	
F Critical one-tail	0.38809002	

Epicatechin **Pass**

F-Test Two-Sample for Variances

	Variable 1	*Variable 2*
Mean	39.9767537	12.9412274
Variance	1689.83276	83.0384956
Observations	19	11
df	18	10
F	20.3499925	
P(F<=f) one-tail	1.4265E-05	
F Critical one-tail	2.79804506	

EGCG **Pass**

F-Test Two-Sample for Variances

	Variable 1	*Variable 2*
Mean	90.27321269	36.488841
Variance	7581.072161	880.922123
Observations	19	11
df	18	10
F	8.605836953	
P(F<=f) one-tail	0.000698632	
F Critical one-tail	2.798045061	

d) T-tests for Fruit vs. No-Fruit

Caffeine **equal variance**

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	100.518697	80.0146218
Variance	2277.00123	1436.15711
Observations	19	11
Pooled Variance	1976.69976	
Hypothesized Mean Difference	0	
df	28	
t Stat	1.21725717	
P(T<=t) one-tail	0.11683326	
t Critical one-tail	1.70113093	
P(T<=t) two-tail	**0.23366652**	
t Critical two-tail	2.04840714	

Catechin **Not pass**

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	20.5720552	13.1369
Variance	123.412222	262.072508
Observations	19	8
Hypothesized Mean Difference	0	
df	10	
t Stat	1.18671279	
P(T<=t) one-tail	0.13138406	
t Critical one-tail	1.81246112	
P(T<=t) two-tail	**0.26276812**	
t Critical two-tail	2.22813885	

Epicatechin **Pass**

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	39.9767537	12.9412274
Variance	1689.83276	83.0384956
Observations	19	11
Hypothesized Mean Difference	0	
df	21	
t Stat	2.75232209	
P(T<=t) one-tail	0.00596845	
t Critical one-tail	1.7207429	
P(T<=t) two-tail	**0.0119369**	
t Critical two-tail	2.07961384	

EGCG **Pass**

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	90.27321269	36.488841
Variance	7581.072161	880.922123
Observations	19	11
Hypothesized Mean Difference	0	
df	24	
t Stat	2.457245917	
P(T<=t) one-tail	0.010804764	
t Critical one-tail	1.71088208	
P(T<=t) two-tail	**0.021609528**	
t Critical two-tail	2.063898562	

V. HPLC results and averages for each selected Kombuchas

a) Fruit-containing kombucha HPLC Results (ppm)

Flavor	Caffeine	Avg Caffeine	Catechin	Avg Catechin	Epicatechin	Avg Epicatechin	EGCG	Avg EGCG
Hibiscous lime 1	27.769	29.790	28.069	24.217	9.460	10.014	5.111	6.012
Hibiscous lime 2	33.883		22.093		10.722		6.912	
Hibiscous lime 3	27.719		22.490		9.859		6.014	
Blackberry ginger day 14	159.027	159.027	49.458	49.458	45.350	45.350	98.901	98.901
Strawberry Rhubarb Ginger 1	86.716	88.578	28.597	27.059	12.711	13.403	30.848	29.970
Strawberry Rhubarb Ginger 2	89.534		27.042		13.483		31.230	
Strawberry Rhubarb Ginger 3	89.485		25.539		14.016		27.834	
Mango ginger	84.401	85.321	15.080	16.504	10.309	10.533	31.806	32.175
mango ginger 2	84.727		16.046		10.311		32.535	
Mango ginger 3	86.836		18.386		10.978		32.184	
Cherry 1	76.085	75.396	1.968	2.081	8.974	9.583	56.118	51.501
Cherry 2	75.358		2.074		9.597		48.201	
Cherry 3	74.745		2.201		10.178		50.186	
Strawberry 1	147.805	167.593	24.877	23.955	96.823	104.358	210.107	230.979
Strawberry 2	204.233		26.685		115.879		259.569	
Strawberry 3	150.741		20.304		100.372		223.261	
Pineapple 1	139.988	136.931	26.003	19.987	90.566	90.179	194.634	188.125
Pineapple 2	135.389		16.148		90.115		185.796	
Pineapple 3	135.415		17.810		89.856		183.945	

b) No-fruit containing kombucha HPLC Results (ppm)

Plain 1	85.351	81.895	15.270	11.598	12.221	9.811	36.418	36.377
Plain 2	77.249		8.285		11.276		36.335	
Plain 3	83.084		11.238		5.938		34.178	
Ginseng-turmeric 1	37.615	32.953	2.870	1.523	11.009	10.969	5.543	4.274
Ginseng-turmeric 2	30.376		1.194		10.648		3.627	
Ginseng-turmeric 3	30.867		0.506		11.250		3.652	
Week 2	158.819	158.819	50.427	50.427	38.351	38.351	109.912	109.912
14 Day plain	94.914	94.914	15.306	15.306	18.793	18.793	39.051	39.051
Lavendar 1	73.599	93.962			6.650	7.622	35.614	44.221
Lavendar 2	104.934				8.076		48.406	
Lavendar 3	103.352				8.141		48.642	