THE DETERMINATION OF ANIONS AND METALS IN KOMBUCHA USING ION CHROMATOGRAPHY AND INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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LIST OF ABBREVIATIONS

EPA	Environmental Protection Agency
HF	Hydrofluoric Acid
HNO_3	Nitric Acid
IC	Ion Chromatography
ICPOES	Inductively Coupled Plasma Optical Emission Spectroscopy
kw	kilowatt
mg/L	Milligrams per Liter
mL	Milliliter
mL/min	Milliliter Per Minute
L	Liters
LOD	Limit of Detection
LOQ	Limit of Quantitation
MΩ	Mega ohm
$NaHCO_3$	Sodium Bicarbonate
Na_2CO_3	Sodium Carbonate
NaF	Sodium Fluoride
NaCl	Sodium Chloride
$NaCH_3COO$	Sodium Acetate
NaBr	Sodium Bromide
$NaNO_2$	Sodium Nitrite
$NaNO_3$	Sodium Nitrate
Na_3PO_4	Sodium Phosphate
Na_2SO_4	Sodium Sulfate
%RSD	Relative Standard Deviation
SCOBY	Symbiotic Culture of Bacteria and Yeast
uL	Micro Liter
USDA FSIS	United States Department of Agriculture Food Safety Inspection Service
VOCs	Volatile Organic Compounds

ABSTRACT

THE DETERMINATION OF ANIONS AND METALS IN KOMBUCHA USING ION CHROMATOGRAPHY AND INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY

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Kombucha is a fermented tea that has been consumed since the time of the Tsin Dynasty in China.[1] It is produced from the modification of sweetened tea by a symbiotic culture of bacteria and yeast. The culture is called a SCOBY and it initiates several transformative chemical processes in sweetened tea that produces ethanol, organic acids, and acetic acid. The purpose of this research project was to develop quality analysis and control methods for a regional kombucha manufacturer. Inorganic and organic ions and metals were determined in finished kombucha, source materials, commercially available teas, and competitor beverages. Sample treatment assays and instrument methods were developed for these compounds. Ion Chromatography (IC) was used to determine organic and inorganic anions while Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) was used to determine metals. Anion concentrations of acetate, chloride, nitrate, phosphate, and sulfate varied between each kombucha flavor. This is likely due to different additives, such as; fruit juice, spices, and extracts. Acetate was found in the highest concentration, which is to be expected because acetic acid is a major by-product of sweet-tea fermentation. Analyses showed that the concentrations of aluminum, calcium, iron, magnesium, manganese, and sodium were relatively consistent across

10 different kombucha flavors. Concentrations in the kombucha flavors were similar to concentrations in the competitor beverages and brewed tea samples. In general, calcium, magnesium, and sodium were the most prevalent metals found in the 10 kombucha flavors.

CHAPTER ONE: INTRODUCTION

The purpose of this thesis project was to develop and perform kombucha quality analysis methods for a regional company. The project consisted of determining compounds of interest in finished kombucha and source materials. This would help the company to gauge product quality and help with identifying new source materials. The compounds of interest included anions and metals. Sample treatment assays and instrument methods were developed. Ion Chromatography (IC) was used to determine organic and inorganic anions while Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) was used to determine metals. For comparative purposes, commercially-available dry raw teas, brewed teas, and competitor brands of kombucha were also analyzed.

In 2007, Americans spent 105 billion dollars on refreshment beverages.[2] Due to market demand and variety, manufacturers strive to produce high-quality and satisfactory products that meet consumers' needs. Manufacturers test their products to make sure they meet specific guidelines. Manufacturers ensure that the concentrations of compounds in their products are within reasonable limits, are consistent from batch to batch, and do not violate patents on other competitive products.[3]

CHAPTER TWO: BACKGROUND, RELATED WORK, AND OBJECTIVES

History

Kombucha is a fermented tea that has been consumed since the time of the Tsin Dynasty in China.[1] It is produced from the modification of sweetened tea by a symbiotic culture of bacteria and yeast known as a SCOBY. The consumption of kombucha dates back several thousand years. Kombucha originated in China in the year 220 B.C. during the time of the Tsin Dynasty where it was used as a detoxifier and energizer.[4] Kombucha was brought to Japan in the year 414 to help the Japanese Emperor with digestive ailments.[4] The tea spread to Russia and Europe in the 20th century by Asian merchants.[4] The popularity of kombucha is growing steadily worldwide especially in the United States since 2000.[5] Kombucha is sold in retail food stores and is often combined with different juices to produce different flavors.

Medicinal Claims

Though kombucha is associated with many positive health effects, the majority of health claims are anecdotal.[6] In 1915, Russian Professor S. Bazarewski reported in the "Correspondence for the Association of Nature Researchers in Riga" that the Latvian population consumed a beverage name "Wonder-mushroom" that helped with headaches and other diseases. Russian Professor Rudolf Kobert reported that kombucha helped with gastric disorders and joint rheumatism in 1918.[1,7] In 1928 Dr. Maxim Bing stated that kombucha helped with high blood pressure.[1,7] In 1929, Russian Doctor, E. Arauner, reported that kombucha helped with diabetes.[1,7] In the 1950s, Russian scientists conducted population studies regarding the cancer benefits of kombucha.[1] Scientists looked at people who lived in areas of Russia that manufactured potassium, lead, mercury, and asbestos.[1] The environmental conditions, age, and everyday habits of the people in these areas were examined. Scientists noticed that the people were drinking kombucha, and attributed this to the low cancer rates. In 1964, Russian Doctor, Rudolf Sklenar, reported that kombucha is a detoxifier that can dissolve cholesterol. He also reported that kombucha can help with cancer in the early stages by balancing the intestinal flora in the stomach.[1] Medical studies conducted by physicians in Germany, Switzerland, and the Netherlands in the 1960s, backed up earlier kombucha health claims made by the Russian scientists.[4]

Production and Chemistry of Kombucha

The process of making kombucha starts with making sweet tea. The tea is transferred to a container and often acidified with vinegar. A SCOBY is added to the tea, covered with a cloth to allow air exchange, and fastened securely. The kombucha is allowed to ferment for up to eight weeks at a temperature range between 20 and 30 ˚C. After fermentation is complete, the SCOBY is removed, and can be stored. Sugar is often added to the kombucha to reactivate the yeast. Bottled kombucha should be stored at 4 ˚C. Figure 1 shows jars of prepared kombucha.

Figure 1. Jars of prepared kombucha.
Source: Slocum, M. How to Make Kombucha http://wholenaturallife.com/how-to-make-kombucha/ (accessed Mar. 25,2016).[8]

The production of kombucha creates two major chemical reactions. First, yeast consumes sugar and produces carbon dioxide and ethanol. Second, acetic acid bacteria (*Acetobacter xylinum*) oxidize ethanol to acetic acid.[9] The production of acetic acid lowers the pH and alcohol content of kombucha. During the fermentation period, the taste of kombucha changes from a sour fruity flavor to a mild vinegar taste. Figure 2 shows relevant chemical reactions.

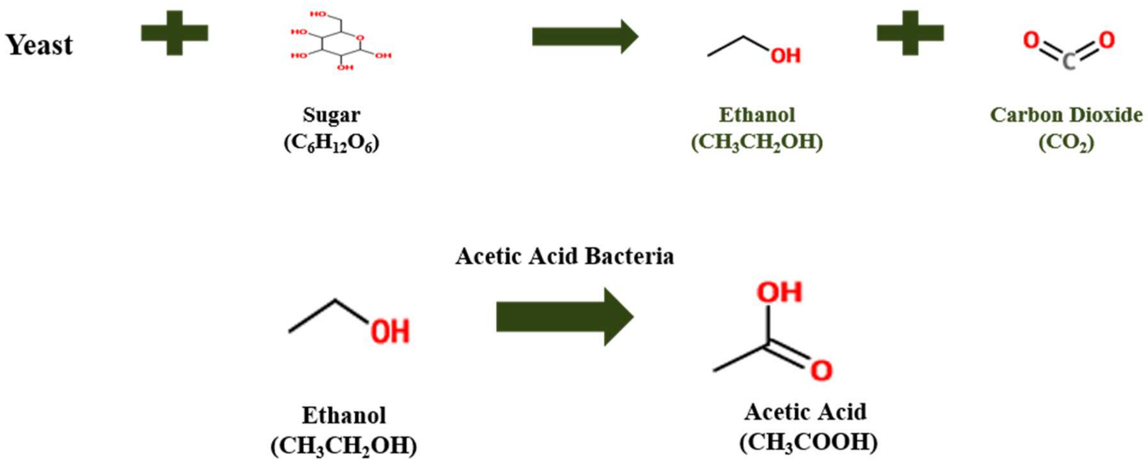


Figure 2. Chemical reactions in kombucha.
Adapted from Greenwalt, C. .; Ledford, R. .; Steinkraus, K. Determination and Characterization of the Antimicrobial Activity of the Fermented Tea Kombucha. *LWT - Food Sci. Technol.* **1998**, *31* (3), 291–296.

Chemical Composition of Kombucha

Kombucha contains anions, metals, organic acids, polyphenols, alkaloids, aroma, and flavor compounds.[3] The most common anions in kombucha are chloride (Cl^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), and acetate (CH_3COO^-).[10,11] The most common metals are aluminum (Al), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), and sodium (Na).[12,13] Anions and metals contribute to kombucha's quality and taste.[3] The most common organic acids are acetic, gluconic, glucuronic, citric, and lactic acid.[14,15] Organic acids contribute to the acidity, aroma, and flavor of kombucha.[3] The most common polyphenols in kombucha are epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate.[16] Alkaloids in kombucha include theobromine, theophylline, and caffeine. Volatile organic compounds (VOCs) like esters, ketones, and aldehydes are present. These compounds also contribute to the aroma and flavor of kombucha.

CHAPTER THREE: EXPERIMENTAL SECTION

Experimental Methods

The focus of this research involves the analysis of several different flavors of kombucha from a regional company. Due to confidentiality, the regional company's kombucha will be referred to as Brand A. Brand A was provided directly from the company in bottles. Source materials like sugar and Humi tea were provided directly from the company in labeled Ziploc bags. Sugar and Humi tea are used to create the sweet tea that will be fermented to create kombucha. Other source materials provided by the company will be referred to as Fermented Tea A, Fermented Tea B, Base Sweet Tea, and Kombucha Al. For comparison, competitor kombuchas and brewed teas were analyzed as reference material. Reference material was necessary because there were few published articles on the determination of anions and metals in kombucha. The competitor kombuchas will be referred to as Brands B and C. Competitor kombuchas, commercially-available black, green, oolong, and herbal raw teas were purchased from local stores.

Materials for IC

- 18.2 Megaohm Ultrapure Water
- Sodium Carbonate
- Sodium Bicarbonate
- Sodium Fluoride
- Sodium Acetate
- Sodium Chloride
- Sodium Nitrite
- Sodium Nitrate
- Sodium Bromide
- Sodium Phosphate
- Sodium Sulfate
- 14.0 mL Conical Tubes
- Dionex ICS 1600

Reagents for IC

A concentrated stock solution of 450.0 mM of Na_2CO_3 (sodium carbonate) and 140.0 mM $NaHCO_3$ (sodium bicarbonate) was purchased from Thermo Fisher Scientific in Suwanee, GA. Standards used on the IC include, NaF (sodium fluoride), NaCl (sodium chloride), $NaCH_3COO$ (sodium acetate), NaBr (sodium bromide), $NaNO_2$ (sodium nitrite), $NaNO_3$ (sodium nitrate), NaH_2PO_4 (monosodium phosphate), and Na_2SO_4 (sodium sulfate). Sodium bromide, chloride, nitrite, and nitrate were certified ACS grade, and purchased from Fisher Scientific in Fairlawn, NJ. Sodium acetate was purchased from Fisher Science education in Nazareth, PA. Sodium fluoride and sulfate were purchased from JT Baker Chemical Company in Phillipsburg, NJ. Sodium phosphate was purchased from Sigma Aldrich Chemical Company in St. Louis, MO.

Standard and Sample Preparation for IC

Standard Preparation

IC standards were prepared by dissolving solid sodium fluoride, sodium acetate, sodium chloride, sodium nitrite, sodium bromide, sodium nitrate, sodium phosphate, and sodium sulfate in one liter of ultrapure water to make a 1000.0 mg/L stock solution. Six dilutions were made from the stock solution to produce six standards ranging in concentration from 0.0 to 100.0 mg/L. Each standard was analyzed four times.

Kombucha and Source Material Preparation

Brands A, B, and C and source material Fermented Tea A, Fermented Tea B, Base Sweet Tea, and Kombucha Al were degassed and stored in plastic bottles. The bottles were previously cleaned with hot, soapy water and rinsed with Deionized water, 30% nitric acid, and 18.2 MΩ ultrapure water. All kombuchas were stored in a freezer at 0 ˚C until analyses. A 10-fold and 100-fold dilution of each kombucha and source material were made with ultrapure water. One gram of sugar was dissolved in ultrapure water and transferred to a conical tube for analysis. All kombuchas and sugars were prepared four times.

Commercially Available Dried Tea and Source Material Humi Tea Preparation

Commercially available tea bags (green, black, oolong, and herbal) and Humi tea were steeped in boiling hot water and were left to cool to room temperature for 45 minutes. Aliquots of each tea were transferred to conical tubes, and stored in a freezer at 0 ˚C until analysis. A 10 and 100-fold dilution were made for each brewed tea sample using ultrapure water. All liquid tea samples were prepared four times. The residual tea bags were dried and stored for later use for ICPOES analysis.

IC Operating Settings

The IC instrument used was a Dionex 1600 Standard Integrating System with Chromeleon 7 software. The following IC instrument parameters were used: isocratic pump, AERS 500 4 mm electrolytic suppresser, DS6 heated digital conductivity detector, AG22 4 x 50 mm guard column, AS22, 4 x 250 mm column at a temperature of 30 ^{0}C, sample injection volume of 25.0 uL, and flow rate of 1.2 mL/min. The mobile phase was prepared by adding 4.0 mL of 140.0 mM sodium bicarbonate and 450.0 mM sodium carbonate to a volumetric flask, and diluting to 2.0 L with 18.2 MΩ ultrapure water. The final concentration was 0.28 mM $NaHCO_3$ (sodium bicarbonate) and 0.9 mM Na_2CO_3 (sodium carbonate). Analysis time was 20 minutes.

Materials for ICPOES

- Concentrated Nitric Acid
- SPEX Multi-element Plasma Standards XRJR 11 and 12
- Acid Digestion Block
- 14.0 mL Conical Tubes

- ICPOES 4300 Optima DV

Reagents for ICPOES

All standards were prepared with 1000.0 mg/L SPEX Multi-element Plasma Standards, XRJR 11 and 12 purchased from SPEX CertiPrep located in Metuchen, New Jersey. Concentrated nitric acid was purchased from Fisher Scientific in Fair Lawn, NJ.

Standard and Sample Preparation for ICPOES

Standard Preparation

Standards for ICPOES were prepared by making a 500.0 mg/L stock solution from a 1000.0 mg/L SPEX multi-element plasma standards XRJR 11 and 12. Five dilutions were made from the 500.0 mg/L stock solution to make five standards with concentrations that range from 0.0 to 10.0 mg/L. Each standard was analyzed in triplicates.

Kombucha, Brewed Tea, and Source Material Preparation

Concentrated nitric acid was added to five milliliters of kombucha, brewed tea, and source material. The acidified kombucha, brewed tea, and source material were heated to 125 ^{0}C on an acid digestion block for two hours. After cooling, a 10-fold and 100-fold dilution were made with ultrapure water. All samples were prepared in triplicate.

Raw Tea Bag Preparation for ICPOES

The raw tea bags left over from the brewed tea (see Commercially Available Dried Tea Preparation) were dried for 24 hours at room temperature. The dried tea bags were transferred to a 50.0 mL conical tube with five milliliters of concentrated nitric acid. The tea bags were digested for 12 hours at room temperature. Twenty five milliliters of ultrapure water was added to each conical tube and inverted to mix. A 10 and 100-fold dilution were made of each. The preparation for all residual tea bags were prepared in triplicate.

ICPOES Operating Settings

The optimum conditions are discussed below. The ICPOES instrument was a Perkin Elmer Optima 4100 DV System with Winlab 32 software. The following ICPOES instrument parameters were used: CoolFlow CFT-75 17.2 ˚C Refrigerated Recirculator, transfer optics, input module, UV/Vis detector, argon gas, plasma gas flow of 15 L/min, auxiliary gas flow of 0.2 L/min, and nebulizer gas flow of 0.8 L/min, power 1300 watts of power, peristaltic pump flow rate of 1.5 mL/min, plasma axial view, and equilibration delay of 15 seconds. Each sample scan was performed in triplicate.

CHAPTER FOUR: THE DETERMINATION OF ANIONS IN KOMBUCHA USING IC

Related Work and Literature Review for IC

The anions selected for analysis were the following: fluoride (F^-), acetate (CH_3COO^-), chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}).

Peer reviewed articles have been published on the determination of anions in kombucha and different brands of teas. Minca et al.[3] and Michalski[17] used IC methods that simultaneously determined five anions (fluoride, chloride, nitrate, phosphate, and sulfate) in different tea infusions in 10 and 15 minutes, respectively. Both IC methods required a dilution with water.

Kumar et al.[10] used an IC method that simultaneously determined seven anions (fluoride, chloride, bromide, nitrate, phosphate, sulfate, and iodide) in black and kombucha tea in 35 minutes. The sample preparation required use of an On Guard-RP cartridge and On Guard P cartridge connected in series to remove interfering compounds and increase the analyte signal.

Dionex ICS 1600 Instrument

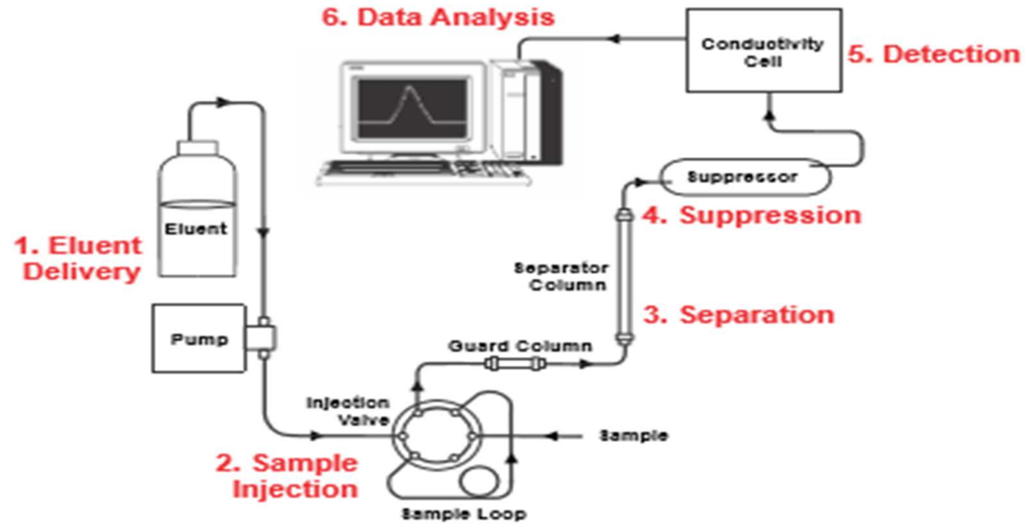


Figure 3. A schematic of the Dionex ICS 1600 instrument.
Source: Reprinted from *Dionex ICS-1600 Ion Chromatography System Operator's Manual,* Copyright 2012 ThermoFisher Scientific Inc.[18]

Figure 3 shows a schematic of the IC instrument. The instrument is composed of six parts: eluent delivery, sample injection, separation, suppression, detection, and data analysis. Eluent is pumped through the sample injection port and onto the guard column where pollutants and contaminants are removed. The eluent is then pumped through the separator column. Sample is injected into the sample loop, which is then introduced into the eluent stream. Once in the eluent stream, the sample is pumped through the guard column and subsequently through the separator column. Once the sample anions elute from the column, the sample anions and eluent enter the suppressor where the conductivity of the eluent is suppressed. After the sample anions leave the suppressor, the detector measures the electrical conductivity for each anion. An IC chromatogram is created of conductivity signal versus retention time.

Anion Exchange Chromatography

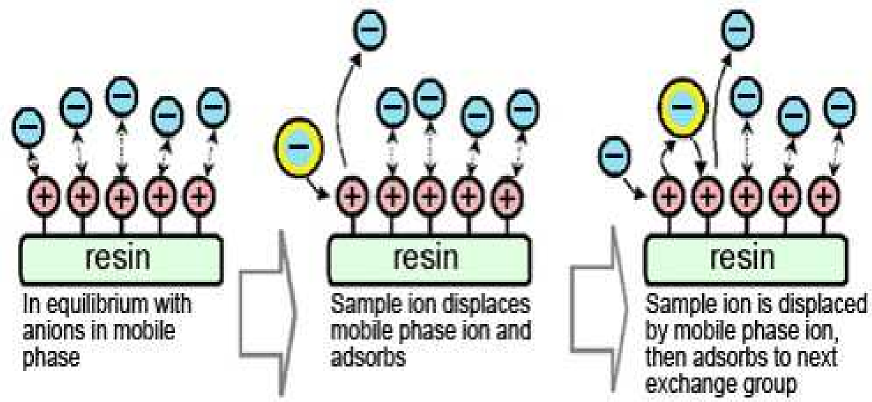


Figure 4. General schematic of anion exchange chromatography.
Source: Inorganic Anion Detection. Shimadzu. http://www.shimadzu.com/an/hplc/support/lib/lctalk/64intro.html. (accessed July 24, 2015).[19]

Figure 4 shows a schematic of anion exchange chromatography. Anion Exchange Chromatography is a separation technique that separates anions based on their differential affinity for an ion exchanger. The ion-exchange column is composed of a resin which has positively charged exchange sites. Anions adsorb to the positive sites. The anionic eluent is passed through the column and dislocates adsorbed anions. When sample is injected into the column, anions will adsorb to the resin until they are dislocated by the eluent and elute from the column. Once eluted from the column, the anions and eluent enter the suppressor.

Suppressed Conductivity Detection

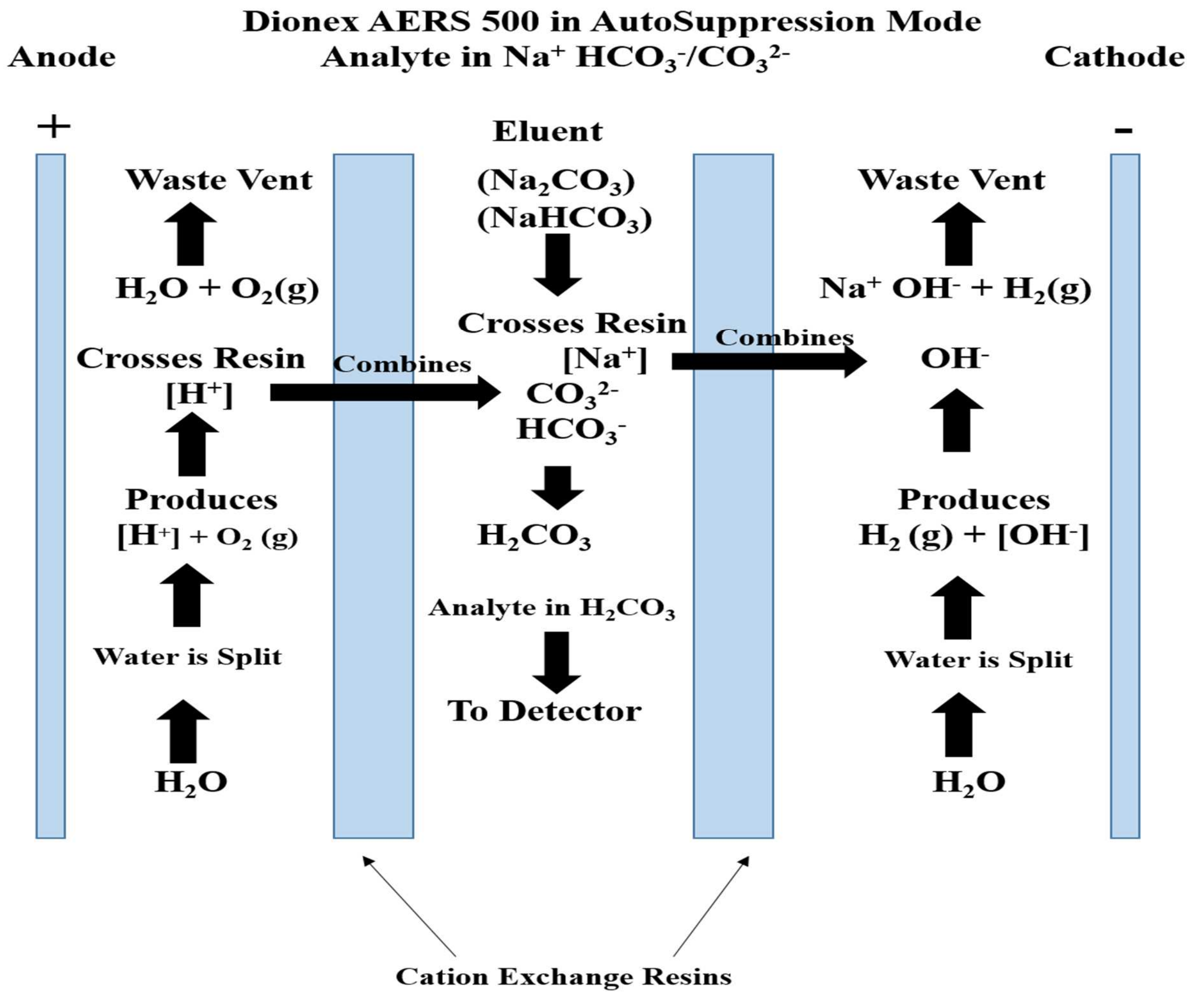


Figure 5. Dionex AERS 500 Auto Suppression.
Source: Adapted from *Dionex ERS 500 Suppressor,* Copyright 2013 Thermo Fisher Scientific Inc.[20]

Figure 5 shows a schematic of the Dionex AERS 500 Auto Suppressor. Suppressors are used in ion chromatography to increase the detector's sensitivity to analytes. This is done by chemically modifying the eluent to reduce the background signal and noise by converting eluent to its neutral form.[21] There is an electric potential that runs from the anode to the cathode chamber. The suppressor is composed of the following three chambers: the anode, eluent, and cathode chamber. In the anode chamber, electrolysis of water forms hydronium ions and oxygen gas. In the cathode chamber, electrolysis of water forms hydroxide ions and hydrogen gas. Both oxygen and hydrogen gas are sent out to waste. The sample anions and eluent flow through the eluent chamber. The sodium cations, due to the electric potential, are pushed towards the cathode chamber where they combine with the hydroxide ions to form base. The hydronium ions in the anode chamber cross through the cation exchange resin where they combine with the eluent anions and sample anions forming their acid counterpart. The hydronium ions also can cross through the cation exchange resin to the cathode chamber to combine with the hydroxide ions forming water .The sample analytes in carbonic acid enter the detector. The pH of the eluent due to carbonic acid is 4.0.[22] According to the Practical Guide to Ion Chromatography, the pKa of carbonic acid is 6.36. At this pKa, the eluent is not dissociated when the eluent reaches the detector. This suppresses the conductivity of the eluent and reduces background noise.

Determining Retention Time of Sample Anions

The retention times were determined as follows: three single element standards (5.0, 50.0, 100.0 mg/L) were made for each of the eight anions (fluoride, acetate, chloride, nitrite, bromide, nitrate, phosphate, and sulfate). IC analysis were performed on all standards to determine retention times. For example, Figure 6 shows an overlay of all three fluoride standards. The 5.0 mg/L standard had a retention time of 3.0 minutes, the 10.0 mg/L had a retention time of 3.05 minutes, and the 100.0 mg/L standard had a retention time of 3.11 minutes. Though there is a slight spread, the range is within typical ranges of retention times.

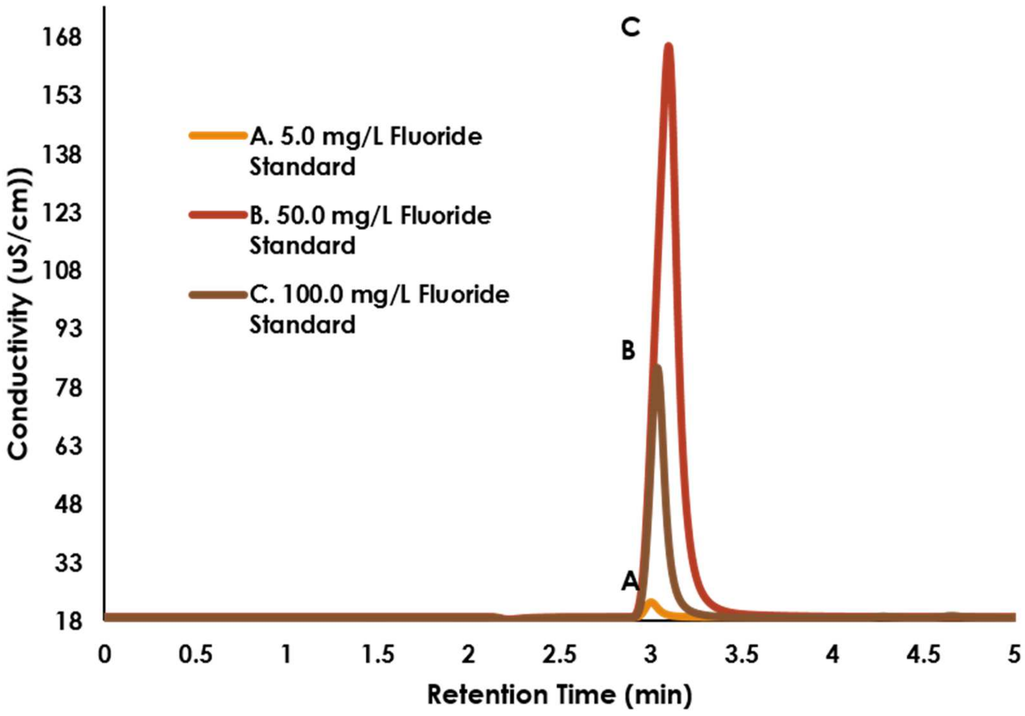


Figure 6. Overlay of three fluoride standards.

Peak Separation of Fluoride and Acetate

In order to check peak resolution among the anions, several mixed standards were analyzed. A 1000.0 mg/L mixed standard was diluted with ultrapure water to obtain a 5.0, 50.0, and 100.0 mg/L mixed standard. Figure 7 shows a chromatogram of the 100.0 mg/L mixed standard. Chloride, nitrite, bromide, nitrate, phosphate, and sulfate peaks are well resolved while the fluoride and acetate peak are not well resolved.

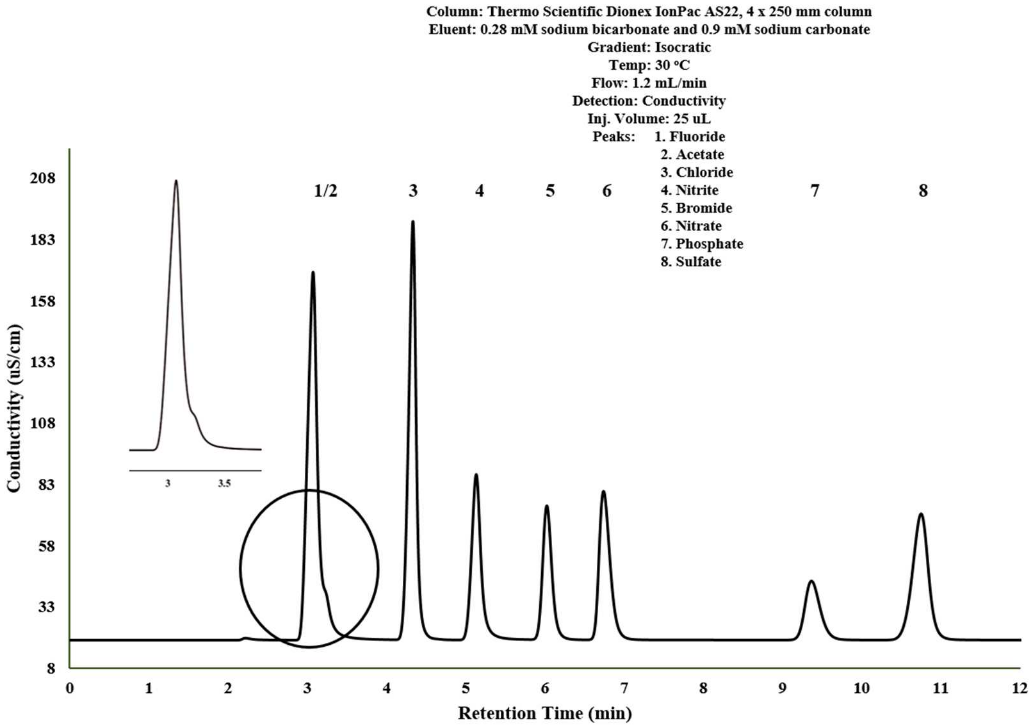


Figure 7. Chromatogram of a 100.0 mg/L mixed standard that has a mobile phase of 1.4 mM sodium bicarbonate and 4.5 mM sodium carbonate. The fluoride and acetate peak are unresolved.

Typically in order to separate fluoride and acetate some parameters are adjusted. For a variety of reasons only the strength of the eluent was changed. The eluent strength was changed by changing the concentration of the mobile phase. The mobile phase was varied until a concentration was found that resolved fluoride and acetate, but did not result in an excessively long analysis time. The optimum concentration was 0.28 mM of sodium bicarbonate and 0.9 mM of sodium carbonate.

Figure 8 shows a 10.0 mg/L mixed standard containing all eight anions. Fluoride and acetate peaks are resolved.

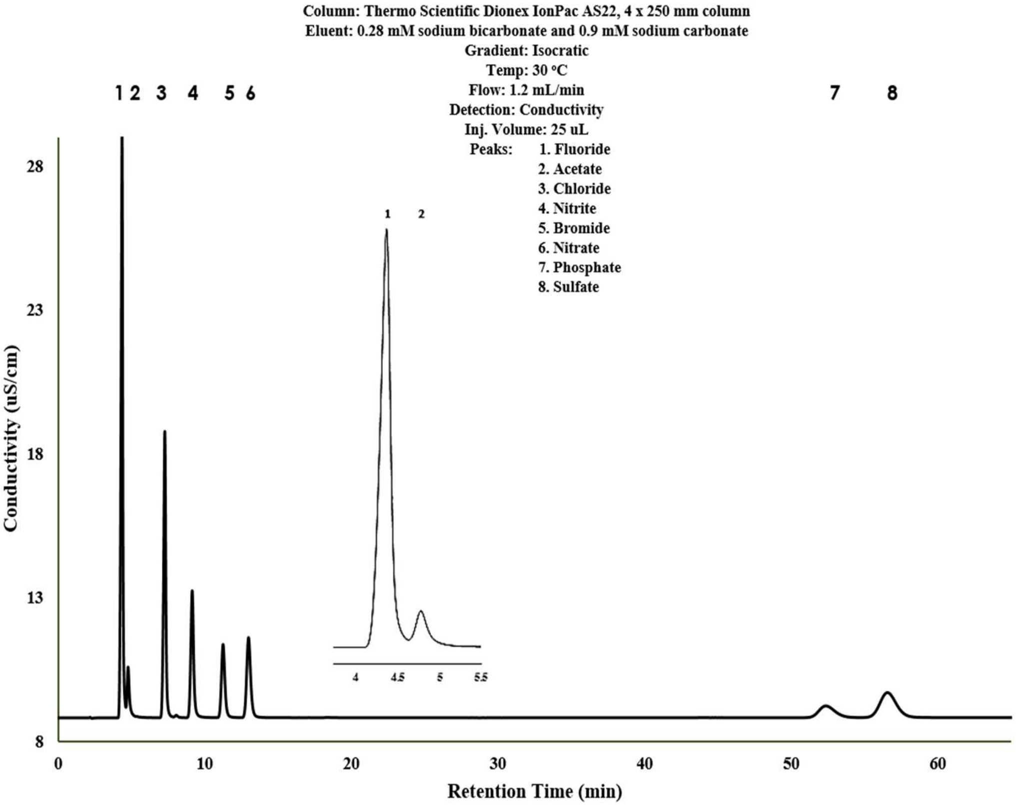


Figure 8. Chromatogram of a 10.0 mg/L mixed standard that has a mobile phase of 0.28 mM sodium bicarbonate and 0.9 mM sodium carbonate. The fluoride and acetate peak are resolved.

Calibration Curve for IC Standards

After optimizing instrument conditions, a calibration curve was created using mixed standards that ranged in concentration from 0.0 mg/L to 100.0 mg/L.

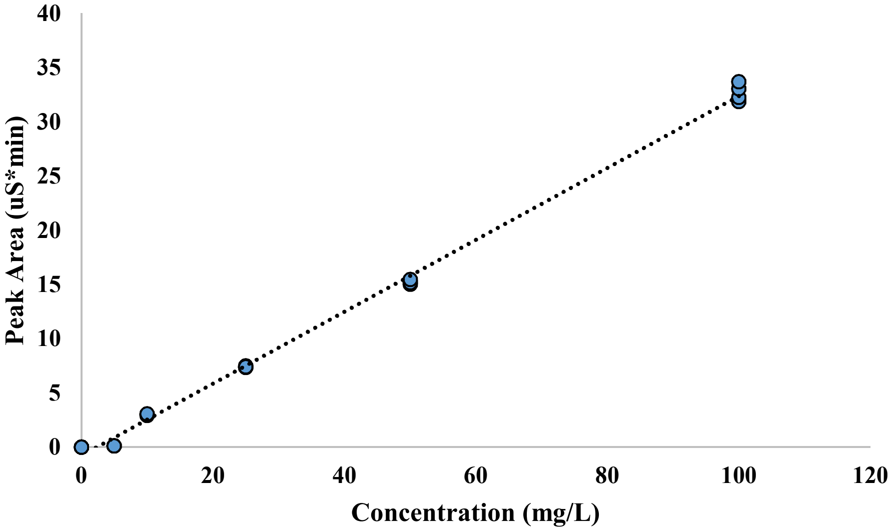


Figure 9. Calibration curve for fluoride. The line of best fit equation is *A = (0.3318 uS*min) C - 0.7716)* with a coefficient of determination of 0.997.

LOD and LOQ

To determine the LOD, the blank standard deviation was multiplied by three and divided by the slope of the line from the calibration curve. To determine the LOQ, the blank standard deviation was multiplied by ten, and divided by the slope of the line from the calibration curve to obtain the LOQ.

Table 1 shows the limit of detection (LOD) and limit of quantitation (LOQ) for the instrument method. The equations used to determine the LOD and LOQ are listed in Figure 10. Ten blanks of ultrapure water were analyzed, and the average peak areas and standard deviations were calculated for eight anions. To determine the LOD, the blank standard deviation was multiplied by three and divided by the slope of the line from the calibration curve. To determine the LOQ, the blank standard deviation was multiplied by ten, and divided by the slope of the line from the calibration curve to obtain the LOQ.

Table 1. The LOD and LOQ in mg/L for eight anions on the IC instrument.

Anions	LOD	LOQ
Fluoride	**0.01**	**0.02**
Acetate	**0.1**	**0.5**
Chloride	**0.03**	**0.1**
Nitrite	**0.01**	**0.03**
Bromide	**0.003**	**0.01**
Nitrate	**0.2**	**0.6**
Phosphate	**0.02**	**0.1**
Sulfate	**0.1**	**0.2**

$$\mathbf{LOD = 3 \; * \; S/m}$$
$$\mathbf{LOQ = 10 * S/m}$$

Figure 10. LOD and LOQ equations. S stands for standard deviation while m stands for slope.
Source: Adapted from Harris, D. C. Quality Assurance and Calibration Methods. In Quantitative Chemical Analysis, 8th ed.; Clancy Marshall: New York, 2010; pp 96-116.[23]

IC Analysis

Sample Comparison to Standard

The Dionex instrument has a retention time window of +/- 5% to allow for shifts in retention times. The IC chromatograms of Brands A, B, and C kombucha and brewed tea were compared to a mixed standard containing all eight anions. All anions identified in the samples fell within the 5% retention time window of the standard containing all eight anions. Figure 11 shows an IC chromatogram of a 10.0 mg/L standard containing all eight anions compared to a

Brand A kombucha sample. The anions determined in the sample were acetate, chloride, nitrate, phosphate, and sulfate. All anions identified in the samples were within this window.

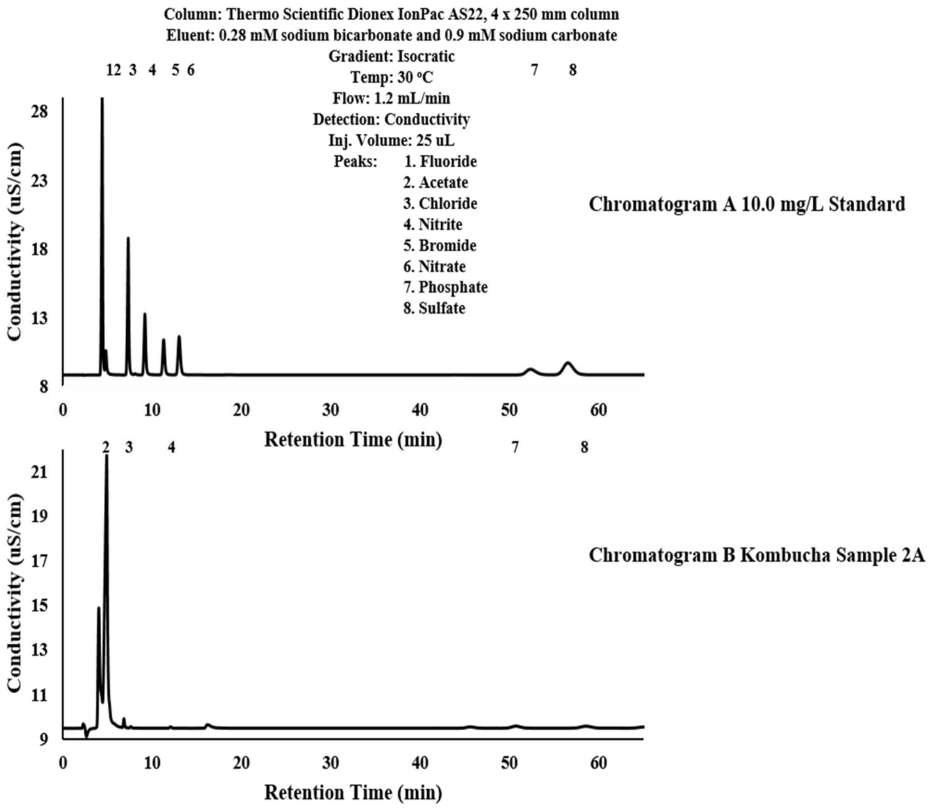


Figure 11. Chromatogram A is a 10.0 mg/L standard containing all eight anions. Chromatogram B is Brand A sample 2A. Anions present are acetate, chloride, nitrate, phosphate, and sulfate.

Retention Time Verification

Retention time verification was done to make sure that the anions being identified in the samples were accurate. Verification was done by spiking a Brand A sample with 10.0 mg/L of chloride, phosphate, and sulfate. Figure 12 shows an overlay of Brand A with no spike, Brand A spiked with 10.0 mg/L of chloride, phosphate, and sulfate, and a 10.0 mg/L mixed standard of all eight anions. Peak identification was confirmed because the peak height of chloride, phosphate, and sulfate increased in the 10.0 mg/L spiked sample when compared to the sample without the spike. The spiked sample did not show additional peaks in the chromatogram. This suggests that the anions identified in Brand A are chloride, phosphate, and sulfate.

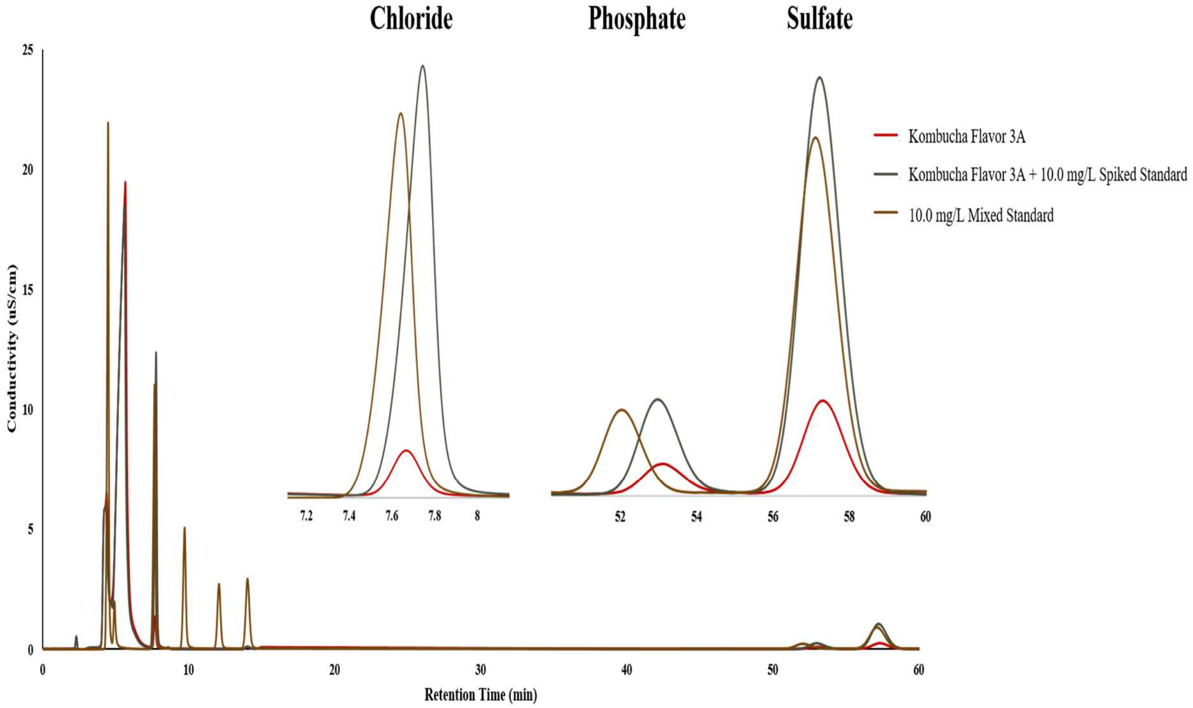
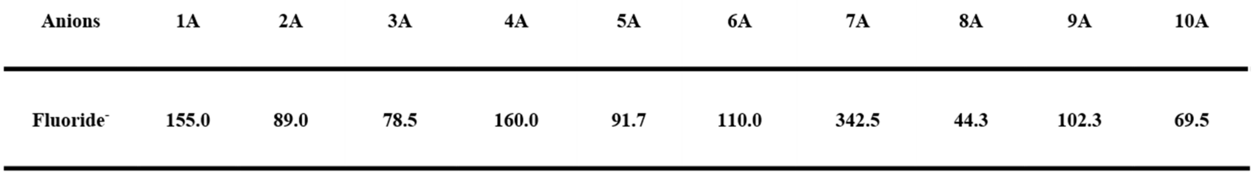


Figure 12. An overlay of Brand A sample 3A, sample 3A spiked with 10.0 mg/L of chloride, phosphate, and sulfate, and a 10.0 mg/L standard containing all eight anions.

Fluoride Determination

The concentrations of fluoride determined in ten of Brand A kombuchas are listed below in Table 2. The high concentrations of fluoride were alarming since they exceeded the Environmental Protection Agency's (EPA) 4.0 mg/L limit in drinking water.[24] To address this potentially serious situation, the method of standard addition was used to confirm fluoride concentration levels found by external calibration. Standard addition is a technique that is used to determine the concentration of analytes in the presence of a complex matrix. Kombucha beverages contain a complex matrix of organic acids, polyphenols, alkaloids, and aroma and flavor compounds that can potentially interfere with the IC performance. This can cause inaccuracies when determining the concentration. Standard addition will increase the instrument response due to the addition of recurring concentrations of analyte standard. The original concentration of the sample analyte can then be determined by plotting the signal versus the concentration. From the trendline and x-intercept, the concentration of fluoride can be more accurately determined.

Table 2. Original concentrations of fluoride in ten of Brand A kombuchas in mg/L.



Anions	**1A**	**2A**	**3A**	**4A**	**5A**	**6A**	**7A**	**8A**	**9A**	**10A**
Fluoride^{-}	**155.0**	**89.0**	**78.5**	**160.0**	**91.7**	**110.0**	**342.5**	**44.3**	**102.3**	**69.5**

Standard addition of fluoride. Five Brand A kombuchas, 3a, 4a, 7a, 9a, and 10a were chosen at random to test if the method of standard addition could show a difference in fluoride concentration. Brand A kombuchas were spiked with varying concentrations of fluoride 0.0, 10.0, 20.0, and 30.0 mg/L to make a linear calibration curve. The standard addition calibration curve for one of the kombuchas, Brand A sample 3a, is presented in Figure 13. The area of the fluoride peak was plotted on the y-axis while the concentration of the fluoride standard was plotted on the x-axis. The concentration of fluoride was determined to be 2.1 mg/L +/- 0.2 mg/L. Brand A kombuchas 4a, 9a, and 10a, had fluoride concentrations below 4.0 mg/L while 7a, had a fluoride concentration greater than 4.0 mg/L (the EPA limit).

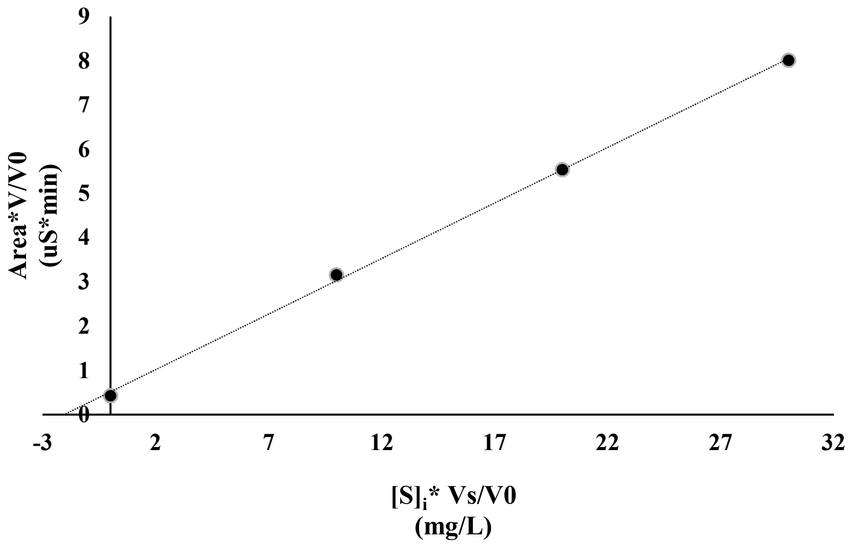


Figure 13. Sodium fluoride standard addition of Brand A sample 3a. The line of best fit equation is *A = (0.251 uS*min) C + 0.5205)* with a coefficient of determination of 0.9992.

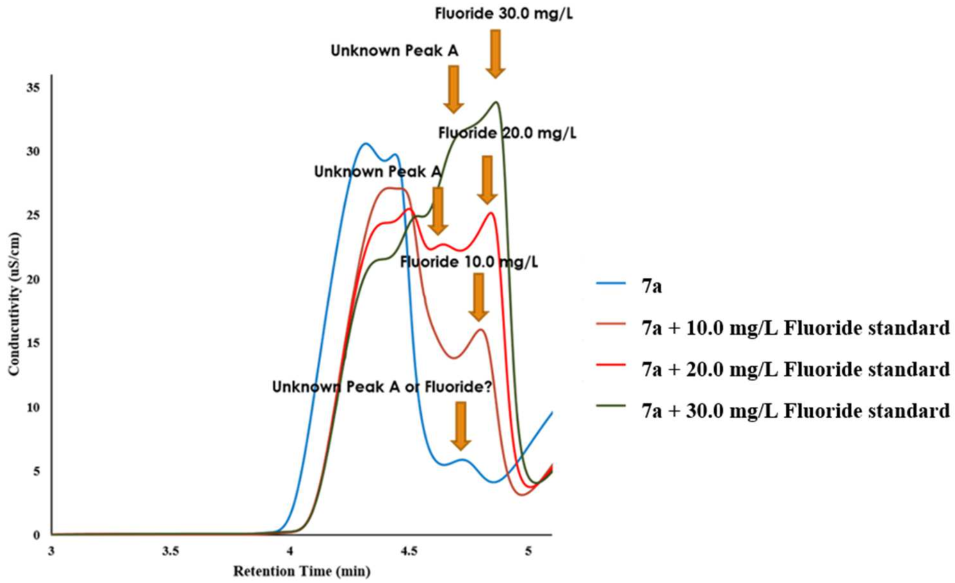


Figure 14. Overlay of IC chromatograms for Brand A sample 7a, 7a with 10.0 mg/L fluoride standard, 7a with 20.0 mg/L fluoride standard, and 7a with 30.0 mg/L fluoride standard.

In spite of the standard additions works, there was still uncertainty about fluoride concentrations. Figure 14 shows an overlay of the IC chromatograms from the standard additions analysis. The chromatogram shows Brand A sample 7a, 7a with 10.0 mg/L fluoride standard, 7a with 20.0 mg/L fluoride standard, and 7a with 30.0 mg/L fluoride standard. There is an increase in the height of one of the peaks as the concentration increases. This peak was identified as fluoride. In 7a with no addition of fluoride there is a peak at 4.75 minutes that is close to the same retention time as the fluoride in the standards. Since this peak is within the 5% retention time window of fluoride, it was determined to be fluoride. However, in 7a with 20.0 and 30.0 mg/L spike of fluoride standard, there was an additional peak noticed to the left of the fluoride peak. This peak also had a similar retention time to the peak in 7a with no addition of fluoride. The peak in 7a at 4.75, which is most likely due to matrix effects, cannot be identified as fluoride due to interferences from the sample matrix. The determination of fluoride was halted because matrix effects make fluoride determination inconclusive.

Impact of organic acids on fluoride determination. Organic acids were analyzed to determine if they impact fluoride determination. The most common organic acids in kombucha are acetic, gluconic, glucuronic, citric, and lactic acid.[14,15] Acetic acid elutes at a later retention time than fluoride. The AS 22 separator column on the Dionex ICS is typically used for low molecular weight organic acids such as acetic acid or formic acid. High molecular weight organic acids are typically analyzed using a different column. Two organic acids were chosen for analysis. Gluconic and glucuronic acid were chosen because they are the two most common organic acids in kombucha.[9] Figure 15 shows an overlay of a 10.0 mg/L standard of gluconic acid, glucuronic acid, and 1.0 mg/L standard of fluoride. All three peaks are resolved in Figure 15, but the matrix of these three standards are aqueous while the matrix of the kombucha samples are more complex. Gluconic acid and glucuronic acid have retention times that fall outside of the 5% retention time window of the fluoride standard. This suggests that gluconic and glucuronic acid may not impact fluoride determination. Further work is required.

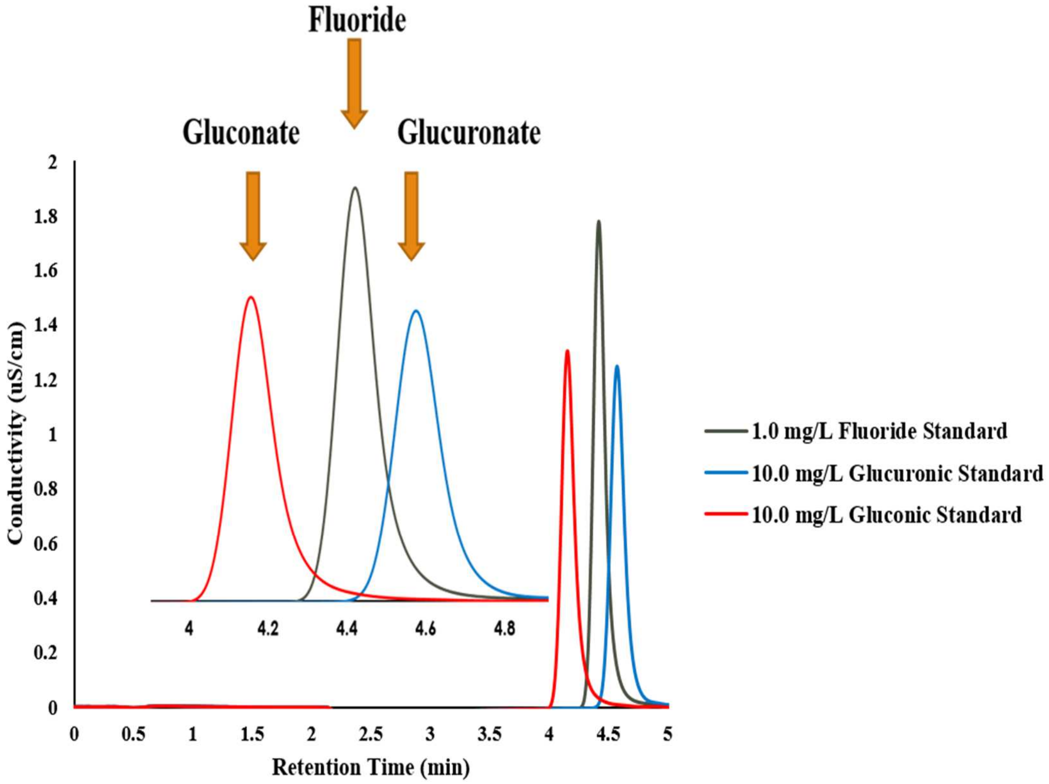


Figure 15. An overlay of a 10.0 mg/L standard of gluconic acid, glucuronic acid, and 1.0 mg/L standard of fluoride.

Impact of ethanol on fluoride determination. The ethanol concentration in kombucha ranges from 0.5 – 3.0% and could contribute matrix interferences with fluoride determination. Six 10.0 mg/L mixed standards containing all eight anions were spiked with ethanol to produce 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0% solutions. According, to Figure 16, peak retention times do not change. This means that ethanol is not responsible for the co-eluting peaks that interfere with fluoride determination. There were also no additional peaks around fluoride peak.

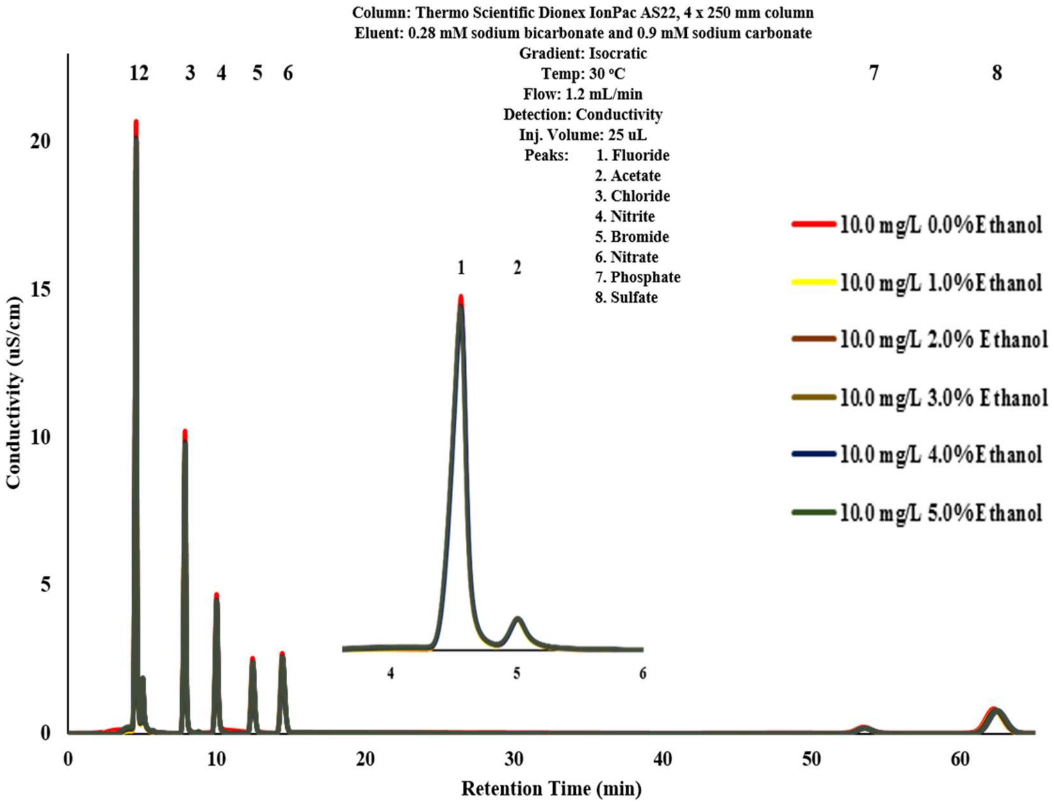


Figure 16. An overlay of six, 10.0 mg/L standards containing all eight anions.

IC Results and Discussion

Kombucha and Brewed Teas

The concentration of anions in kombucha for Brands A, B, and C and brewed teas are listed below in Table 3 and Table 4. Any anions with concentrations below the limit of quantitation are listed as (bql). Fluoride was not determined because of matrix interferences. Nitrite and bromide concentrations were below the level of quantitation.

Table 3. Concentration of anions in mg/L and %RSD in 10 different flavors of Brand A kombucha. *K stands for kombucha. BA stands for Brand A, and a, b, c refers to same flavor different batch.

Samples	Acetate	Chloride	Nitrate	Phosphate	Sulfate
BA1a^{K}	2823.9 (20.1%)	39.0 (4.2%)	bql	54.5 (15.4%)	43.8 (8.4%)
BA1b^{K}	1618.3 (7.2%)	34.7 (3.7%)	bql	60.8 (29.7%)	44.4 (6.7%)
BA2a^{K}	1970.7 (0.3%)	38.6 (0.6%)	43.7 (0.3%)	55.8 (2.2%)	43.3 (2.3%)
BA2b^{K}	2221.3 (6.3%)	36.0 (3.1%)	39.9 (5.8%)	40.9 (5.8%)	44.8 (7.0%)
BA2c^{K}	3790.2 (9.3%)	22.8 (0.9%)	26.4 (3.4%)	36.9 (12.5%)	41.8 (6.6%)
BA3a^{K}	4838.0 (31.2%)	43.8 6.8%)	46.8 (3.9%)	74.0 (21.0%)	47.6 (12.2%)
BA3b^{K}	3497.5 (6.1%)	38.7 (2.9%)	43.8 (1.6%)	53.9 (2.6%)	55.9 (2.6%)
BA3c^{K}	2676.2 (3.5%)	38.3 (6.6%)	42.8 (0.8%)	52.1 (4.6%)	54.9 (6.4%)
BA3d^{K}	2020.9 (14.8%)	34.5 (1.7%)	41.3 (2.5%)	43.6 (6.8%)	45.6 (6.3%)
BA4a^{K}	1550.9 (9.0%)	48.8 (12.5%)	42.9 (0.8%)	40.8 (5.1%)	52.9 (15.9%)
BA4b^{K}	2365.5 (23.8%)	40.3 (7.4%)	bql	bql	45.5 (3.1%)
BA4c^{K}	956.3 (48.5%)	39.6 (0.8%)	bql	bql	47.9 (1.2%)
BA4d^{K}	2409.2 (12.7%)	40.6 (6.0%)	bql	68.2 (12.4%)	38.9 3.0%)
BA5a^{K}	1267.5 (2.9%)	39.0 (0.3%)	bql	53.9 (4.8%)	50.6 (5.0%)
BA6a^{K}	1301.4 (11.3%)	41.0 (2.2%)	42.3 (0.2%)	70.8 (4.9%)	63.9 (6.0%)
BA7a^{K}	1791.7 (20.5%)	214.4 (19.7%)	42.3 (0.4%)	94.9 (14.4%)	140.5 (17.2%)
BA8a^{K}	813.4 (4.3%)	42.5 (3.9%)	43.4 (0.7%)	48.5 (7.7%)	35.4 (2.6%)
BA9a^{K}	2171.2 (14.0%)	124.4 (21.1%)	43.0 (0.5%)	62.4 (12.9%)	48.4 (11.3%)
BA9b^{K}	2218.7 (6.4%)	183.0 (11.3%)	bql	58.3 (10.6%)	53.5 (5.2%)
BA9c^{K}	676.8 (5.3%)	134.0 (11.4%)	bql	46.3 (9.5%)	46.2 (6.3%)
BA10a^{K}	988.0 (6.8%)	82.9 (5.2%)	42.8 (4.8%)	54.9 (4.7%)	69.2 (5.5%)
BA10b^{K}	3289.1 (8.2%)	207.0 (3.8%)	40.1 (0.2%)	68.9 (1.6%)	57.5 (1.8%)

Table 4. Concentration of anions in mg/L and %RSD in four flavors of Brand B kombucha, five flavors of Brand C kombucha, and brewed teas. *BT stands for black tea, GT stands for green tea, OT stands for oolong tea, HT stands for herbal tea, and KT standards for kombucha tea.

Names	Acetate	Chloride	Nitrate	Phosphate	Sulfate
Ceylon Orange Pekoe^{BT}	bql	29.0 (2.0%)	bql	19.6 (10.8%)	34.6 (3.1%)
Chai French Vanilla^{BT}	bql	30.2 (3.1%)	bql	21.5 (1.7%)	35.6 (1.2%)
Darjeeling^{BT}	bql	20.6 (0.8%)	22.3 (0.2%)	20.0 (2.7%)	25.1 (0.7%)
Lapsang^{BT}	bql	20.8 (0.4%)	22.4 (0.3%)	21.7 (2.6%)	25.7 (1.7%)
Oolong^{OT}	bql	20.8 (1.0%)	22.3 (0.2%)	18.0 (2.5%)	27.5 (2.4%)
Prince of Whales^{BT}	bql	20.8 (1.0%)	22.5 (0.5%)	18.6 (4.5%)	26.5 (1.7%)
Green^{GT}	bql	27.8 (1.1%)	bql	22.0 (2.5%)	35.9 (0.7%)
Yaupon^{HT}	bql	34.6 (1.3%)	bql	115.8 (7.7%)	31.2 (2.4%)
Tony^{HT}	bql	30.7 (3.2%)	bql	28.4 (43.7%)	28.0 (1.3%)
Gyokuro^{GT}	bql	41.4 (1.3%)	43.1 (0.8%)	194.4 (3.6%)	62.0 (1.5%)
BB1a^{K}	1146.8 (50.3%)	25.7 (1.6%)	23.0 (0.3%)	54.2 (3.5%)	39.5 (3.1%)
BB2a^{K}	1927.3 (20.3%)	31.6 (1.5%)	22.5 (0.5%)	30.5 (3.8%)	188.0 (5.4%)
BB3a^{K}	864.1 (0.6%)	30.3 (6.7%)	23.3 (0.2%)	27.2 (13.6%)	157.6 (8.4%)
BB3b^{K}	1495.4 (11.1%)	31.4 (1.0%)	23.3 (0.9%)	25.0 (12.4%)	145.5 (9.2%)
BB4a^{K}	3911.7 (6.2%)	130.1 (1.4%)	73.2 (0.6%)	217.7 (9.1%)	411.3 (0.9%)
BC1a^{K}	3177.6 (4.8%)	35.5 (7.9%)	47.6 (12.1%)	160.2 (11.5%)	42.0 (4.2%)
BC2a^{K}	2964.1 (1.5%)	32.9 (2.1%)	62.9 (8.5%)	49.6 (3.9%)	35.9 (0.7%)
BC3a^{K}	2241.2 (5.2%)	33.1 (4.6%)	41.6 (0.3%)	126.8 (1.4%)	39.3 (2.9%)
BC4a^{K}	2979.2 (4.1%)	37.2 (2.7%)	43.2 (4.9%)	43.2 (4.9%)	90.2 (4.4%)
BC5a^{K}	133.4 (18.2%)	35.6 (4.5%)	41.1 (0.1%)	20.7 (8.7%)	30.3 (2.2%)

Anion concentrations of acetate, chloride, nitrate, phosphate, and sulfate in Brand A kombucha varied between each kombucha flavor, but were similar to the concentrations in Brand B and Brand C. This is likely due to the different additives such as fruit juice, spices, and extracts added to the kombucha. Acetate was found in the highest concentration, which is to be expected because acetic acid is a major by-product of sweet-tea fermentation.

Concentration Ranges of Anions

The concentration range of anions for Brands A, B and C kombucha and brewed tea were compiled in Table 5. Fluoride concentrations were not determined. Nitrite and bromide were below the quantitation limit. Acetate concentrations were below the quantitation limit for the brewed tea. Anions below the quantitation limit are listed as bql.

Table 5. Concentration ranges of anions in Brands A, B and C kombucha and brewed tea.

Names	Acetate	Chloride	Nitrate	Phosphate	Sulfate
Brand A	677.0 - 4838.0 mg/L	20.0 - 214.0 mg/L	26.0 – 47.0 mg/L	29.0 - 95.0 mg/L	41.0 - 140.0 mg/L
Brewed Tea	bql	20.0 - 41.0 mg/L	22.0 - 43.0 mg/L	18.0 - 194.0 mg/L	25.0 - 62.0 mg/L
Brand B	864.0 – 3912.0 mg/L	26.0 – 130.0 mg/L	22.0 – 73.0 mg/L	25.0 – 218.0 mg/L	39.0 – 411.0 mg/L
Brand C	133.0 – 3178.0 mg/L	33.0 – 37.0 mg/L	41.0 – 48.0 mg/L	21.0 – 160.0 mg/L	30.0 – 90.0 mg/L

The concentration range of acetate in Brand A were similar to Brand B and Brand C. The range in Brand A was from 677.0 – 4838.0 mg/L while in Brand B the concentration range was 864. 0 mg/L – 3912.0 mg/L and in Brand C 133.0 – 3178.0 mg/L. This is likely due to the acetic acid bacteria converting the ethanol to acetic acid during the fermentation process.

The concentration ranges of chloride, nitrate, phosphate, and sulfate in Brand A were similar to the concentration ranges in Brand B and C. Brand A had chloride concentrations between 20.0 mg/L and 214.0 mg/L while Brand B had chloride concentrations between 6.0 and 130.0 mg/L and in Brand C 33.0 and 37.0 mg/L. Brand A had nitrate concentrations between

26.0 mg/L and 47.0 mg/L while Brand B had nitrate concentrations between 22.0 and 73.0 mg/L and in Brand C 41.0 and 48.0 mg/L. Brand A had phosphate concentrations between 29.0 mg/L and 95.0 mg/L while Brand B had phosphate concentrations between 25.0 and 218.0 mg/L and in Brand C 21.0 and 160.0 mg/L. Brand A had sulfate concentrations between 41.0 mg/L and 140.0 mg/L while Brand B had sulfate concentrations between 39.0 and 411.0 mg/L and in Brand C 30.0 and 90.0 mg/L. Chloride, nitrate, phosphate, and sulfate in the brewed tea were within the ranges of Brand A, B, and C.

IC Analysis of Water

Brand A's source water and WCU's Natural Science Room 217 ultrapure water used in all dilutions and standards were analyzed to determine if they were sources of anions that were observed in Brand A. Table 6 shows the concentrations in Brand A's water and WCU's Natural Science Room 217 water. Acetate, nitrite, bromide, nitrate, and phosphate were below limit of quantitation. Sulfate was below the limit of quantitation for the Natural Science ultrapure water.

Table 6. Concentration of anions in mg/L and %RSD of the company's water and the Natural Science ultrapure water.

Names	Fluoride	Chloride	Sulfate
Brand A Water	0.3 (2.4%)	3.2 (6.6%)	7.4 (17.5%)
WCU NS 217 Ultrapure Water	0.3 (0.2%)	2.5 (0.0%)	

Chloride concentrations were lower in Brand A's water and WCU's Natural Science room 217 water when compared to Brand A kombucha in Table 3. The chloride concentration was 3.2 mg/L in the company water, and 2.5 mg/L in Natural Science water. Both of these concentrations fall below Brand A's chloride concentration range in Table 5. The sulfate concentration in the company’s water was also low. The sulfate concentration was 7.4 mg/L, which falls below Brand A’s sulfate concentration range. The low concentrations of chloride and sulfate in the company water and the low concentration of chloride in Natural Science water, suggests that neither the company water nor Natural Science water, are responsible for the concentrations observed in Brand A kombucha.

IC Analysis of Source materials

Sugar, Humi tea, Fermented tea A, Fermented tea B, Kombucha Al, and Base Sweet Tea anion concentrations are listed in

Table 7. Acetate was below the limit of quantitation for the sugar and Humi tea. Nitrite, and bromide were below limit of quantitation. Anions that had concentrations below the instrument’s quantitation limit are labeled as (bql). Fluoride was not determined.

Table 7. Concentration of anions in mg/L and %RSD of the company’s source materials. *SM stands for source material.

Source Material	Fluoride	Acetate	Chloride	Nitrate	Phosphate	Sulfate
Sugar^{SM}	0.3 (0.1%)	bql	2.5 (0.2%)	Bql	bql	2.7 (0.5%)
Humi Tea^{SM}	***	bql	34.3 (1.9%)	42.6 (3.6%)	162.1 (11.8%)	74.8 (3.5%)
Fermented Tea A^{SM}	***	3502.0 (9.6%)	36.0 (2.7%)	Bql	bql	47.0 (3.5%)
Fermented Tea B^{SM}	***	3952.1 (7.4%)	33.4 (1.6%)	bql	bql	49.4 (1.1%)
Kombucha Al^{SM}	***	2977.4 (4.3%)	36.2 (4.8%)	bql	41.7 (2.5%)	45.0 (1.8%)
Base Sweet Tea^{SM}	***	8.0 (10.2%)	34.7 (0.9%)	bql	29.0 (1.0%)	46.4 (0.9%)

Chloride concentrations were lower in the sugar when compared to Brand A kombucha in Table 3. The sulfate concentrations were also lower in the sugar when compared to Brand A. The chloride concentration was 2.5 mg/L. The sulfate concentration was 2.7 mg/L in the sugar.

The concentrations of chloride, nitrate, phosphate, and sulfate in the Humi tea are similar to the concentrations in Brand A kombucha. The chloride concentration was 34.3 mg/L, the nitrate concentration was 42.6 mg/L, the phosphate concentration was 1621.1 mg/L, and the sulfate concentration was 74.8 mg/L. These concentrations fall within the concentration ranges of chloride, nitrate, phosphate, and sulfate in Brand A in Table 5.

The concentrations of chloride in fermented tea A was 36.0 mg/L, fermented tea B was 33.4 mg/L, kombucha al was 36.2 mg/L, and base sweet tea was 34.7mg/L. These concentrations were within the concentration ranges of Brands A, B, and C. Acetate concentrations in fermented tea A was 3502.0 mg/L, fermented tea B was 3952.1 mg/L, and kombucha al was 2977.4 mg/L. These concentrations were within the concentration ranges of acetate in Brands A, B, and C kombuchas. Base sweet tea acetate concentration was 8.0 mg/L which falls below the concentration ranges of Brand A, B, and C. Phosphate concentrations in kombucha al was 41.7 mg/L and 29.0 mg/L in base sweet tea. These concentrations were within the concentration ranges of Brands A, B, and C. The concentrations of sulfate in fermented tea A was 47.0 mg/L, fermented tea B was 49.4 mg/L, kombucha al was 45.0 mg/L, and base sweet tea was 46.4 mg/L. These concentrations were within the concentration ranges of acetate in Brands A, B, and C.

CHAPTER FIVE: THE DETERMINATION OF METALS IN KOMBUCHA USING ICPOES

Related Work and Literature Review for ICPOES

The metals selected for analysis were silver (Ag), aluminum (Al), barium (Ba), beryllium (Be), bismuth (Bi), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), Vanadium (V), and zinc (Zn).

Peer reviewed journal articles have been published on the determination of metals in kombucha, teas, coffees, and juices. Szymczycha-Madeja et al. [25,13] used ICPOES methods that allowed for the simultaneous determination of 14 metals (Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Sr, and Zn) in coffee. Coffee samples were prepared using microwave and hot plate digestion.

Lara et al.[26], used an ICPOES method that allowed for the simultaneous determination of eight metals (Al, Ca, Cr, Cu, Fe, Ni, Pb, and Zn) in wine. Wine samples were prepared by adding perchloric acid and nitric acid to the samples then heating until dryness. The residue was dissolved with concentrated nitric acid, and diluted with water. The USDA FSIS[27], used an ICPOES method that simultaneously determined 10 metals (Fe, Zn, Cu, Ni, Al, B, Ba, Cr, V, and Sr) in various samples. Samples were prepared using a microwave.

Perkin Elmer 4300 Optima DV

A schematic of the ICPOES-OES 4300 DV is shown in Figure 17.

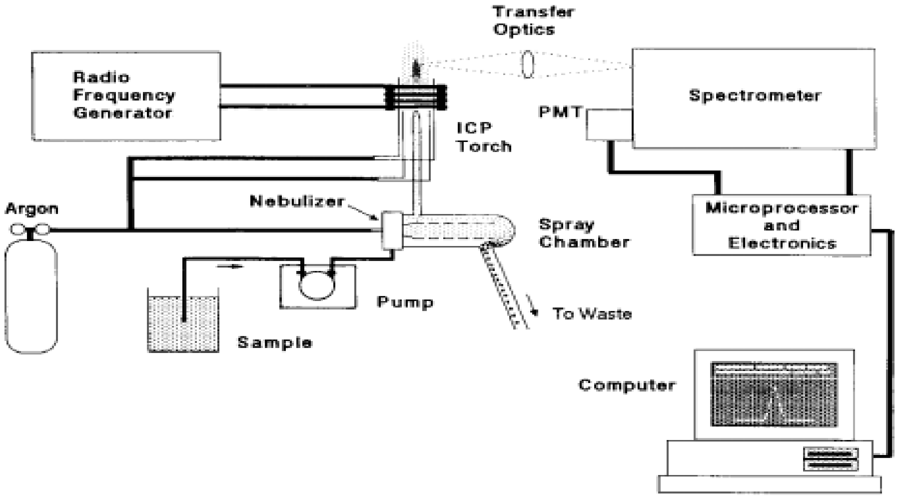


Figure 17. A schematic diagram of the ICPOES 4300 DV.
Source: Reprinted from Ji, Y.-Q.; Adelhelm, C.; Koster, R. Wissenschaftliche Berichte – Forschungszentrum Karlsruhe 2002, 4300 (FZKA 6769), I – iii,1–18.[28]

Inductively Coupled Plasma-Optical Emission Spectroscopy

ICPOES is an instrument used to measure the emission of trace metals in a sample using an inductively coupled plasma. Sample is introduced into a nebulizer where it is turned into a mist. The mist enters into an argon plasma which is surrounded by a radio frequency coil. [29]A radio frequency energy is applied to the coil as argon is passed through the torch. This energy causes electrons to be stripped from argon forming a plasma.[29] Analytes in the sample collide with the plasma, which is composed of electrons and charged ions. The collision atomizes the analytes in the sample. Metal atoms in the sample are excited to a higher energy state, and emit photons. As the molecule return to the ground state, light is emitted, and enters ICPOES. The ICPOES is composed of prisms and gratings that separate the different wavelengths of light so that the wavelengths are distinguishable when they reach the detector.[30] The intensity of the light indicates how concentrated each metal is while the wavelength indicates what metal is present. Each metal can have one or more emission wavelengths. The wavelengths are usually resolved, but sometimes can overlap with other metals' emission wavelengths. If the wavelengths overlap, a different emission wavelength is chosen.

Calibration Curve for ICPOES Standards

A calibration curve was determined using mixed standards that ranged in concentration from 0.0 mg/L to 10.0 mg/L.

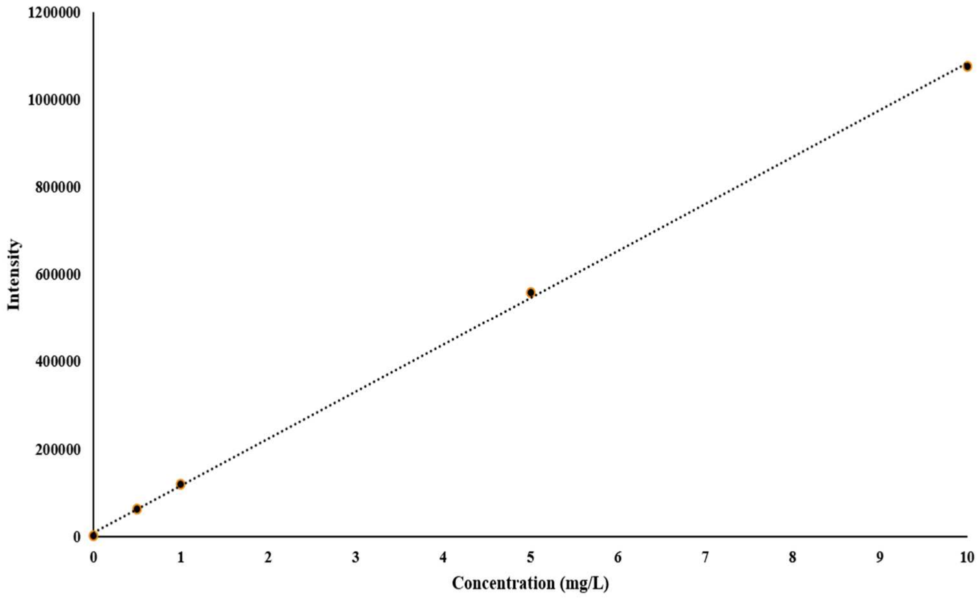


Figure 18. Calibration Curve for Silver (Ag). The line of best fit equation is $I = 107464\,C - 8565.1$ with a coefficient of determination of 0.9997.

LOD and LOQ

Table 8 shows the limit of detection (LOD) and limit of quantitation (LOQ) for the instrument method. The equations used to determine the LOD and LOQ are listed in Figure 10. Ten blanks of ultrapure water were analyzed, and the average peak intensities and standard deviations were calculated for 18 metals. To determine the LOD, the blank standard deviation was multiplied by three and divided by the slope of the line from the calibration curve. To determine the LOQ, the blank standard deviation was multiplied by ten, and divided by the slope of the line from the calibration curve to obtain the LOQ.

Table 8. ICPOES Emission lines, LOD, and LOQ for the multi-elemental determination of 18 metals.

Metals	Wavelength (nm)	LOD	LOQ
Ag	328.1	0.0003	0.001
Al	308.2	0.01	0.04
Ba	233.5	0.0003	0.001
Be	313.1	0.0005	0.002
Bi	223.1	0.003	0.01
Ca	317.9	0.003	0.01
Cd	228.8	0.001	0.003
Co	228.6	0.001	0.003
Cr	267.7	0.001	0.002
Cu	327.4	0.001	0.003
Fe	238.2	0.002	0.006
Mg	285.2	0.001	0.002
Mn	257.6	0.0001	0.0005
Na	330.2	0.01	0.03
Ni	231.6	0.001	0.003
Pb	220.4	0.01	0.02
V	290.9	0.001	0.002
Zn	206.2	0.001	0.003

ICPOES Results and Discussion

Kombucha and Brewed Teas

The metal concentrations for Brands A, B, C, and brewed teas are listed in Table 9 and Table 10. Silver, beryllium, bismuth, cadmium, cobalt, chromium, lead, vanadium, and zinc were below the limit of quantitation. Barium, copper, and nickel had concentrations <0.1 mg/L. Metals that had concentrations below the instrument's quantitation level are labeled as (bql). Metals with concentration values less than 0.1 mg/L are listed as <0.1.

Table 9. Concentration of metals in mg/L and %RSD of 10 different flavors of Brand A kombucha. **K stands for kombucha. BA stands for Brand A, and a, b, c refers to same flavor different batch.

Brand A	Al	Ba	Ca	Cu	Fe	Mg	Mn	Na
BA1a^{K}	1.2 (5.31%)	bql	39.3 (1.2%)	bql	<0.1	4.4 (5.4%)	0.3 (6.7%)	3.4 (5.9%)
BA1b^{K}	1.1 (3.53%)	bql	33.7 (0.8%)	bql	<0.1	5.0 (7.7%)	0.3 (10.0%)	5.4 (5.5%)
BA2a^{K}	1.3 (3.95%)	<0.1	41.6 (3.3%)	bql	<0.1	17.4 (1.8%)	0.7 (3.5%)	4.1 (2.9%)
BA2b^{K}	1.5 (2.8%)	bql	8.9 (4.9%)	bql	bql	7.3 (7.0%)	0.4 (8.5%)	5.0 (1.9%)
BA2c^{K}	1.2 (19.0%)	bql	39.1 (13.1%)	<0.1	0.2 (28.6%)	21.0 (24.2%)	0.7 (0.0%)	5.1 (0.6%)
BA3a^{K}	1.4 (9.4%)	<0.1	39.3 (4.1%)	bql	0.1 (51.7%)	17.5 (3.8%)	0.8 (33.9%)	5.9 (2.7%)
BA3b^{K}	1.2 (38.7%)	bql	8.9 (20.2%)	bql	bql	5.7 (20.7%)	0.4 (26.4%)	4.2 (16.9%)
BA3c^{K}	1.7 (8.0%)	bql	18.2 (1.6%)	bql	<0.1	7.4 (11.5%)	0.6 (17.4%)	5.9 (5.2%)
BA3d^{K}	1.6 (2.9%)	bql	25.0 (0.6%)	bql	<0.1	9.4 (1.6%)	0.8 (2.2%)	6.7 (5.0%)
BA4a^{K}	1.2 (1.7%)	<0.1	45.0 (1.7%)	bql	<0.1	6.8 (15.1%)	0.4 (18.5%)	4.0 (12.2%)
BA4b^{K}	1.3 (3.4%)	bql	17.6 (1.7%)	bql	bql	3.4 (15.7%)	0.2 (26.6%)	4.6 (3.2%)
BA4c^{K}	1.5 (0.7%)	bql	28.0 (1.0%)	bql	bql	5.2 (7.3%)	0.3 (10.4%)	5.1 (5.0%)
BA4d^{K}	1.2 (4.9%)	bql	47.2 (1.4%)	bql	<0.1	7.8 (11.4%)	0.5 (15.3%)	6.2 (3.9%)
BA5a^{K}	1.3 (0.9%)	<0.1	47.4 (1.8%)	bql	<0.1	8.3 (4.5%)	0.3 (5.0%)	4.1 (4.9%)
BA6a^{K}	1.3 (11.8%)	<0.1	48.0 (2.6%)	bql	<0.1	14.9 (2.6%)	0.4 (24.9%)	5.0 (19.5%)
BA7a^{K}	1.3 (5.1%)	<0.1	54.8 (1.7%)	bql	<0.1	20.2 (1.6%)	0.5 (12.4%)	13.5 (1.8%)
BA8a^{K}	1.5 (7.0%)	<0.1	49.8 (1.0%)	bql	0.3 (9.4%)	23.0 (1.0%)	0.5 (10.6%)	9.3 (9.0%)
BA9a^{K}	1.2 (11.0%)	bql	39.8 (0.3%)	bql	<0.1	26.7 (6.1%)	0.7 (38.9%)	15.2 (5.8%)
BA9b^{K}	1.8 (0.7%)	bql	61.9 (0.9%)	bql	bql	26.7 (0.7%)	0.4 (11.7%)	24.8 (0.8%)
BA9c^{K}	1.5 (4.7%)	bql	47.6 (0.9%)	bql	<0.1	0.2 (0.9%)	0.3 (10.5%)	0.2 (0.9%)
BA10a^{K}	1.4 (13.7%)	<0.1	75.9 (0.4%)	bql	0.2 (67.1%)	32.5 (0.5%)	0.7 (11.2%)	17.6 (0.3%)
BA10b^{K}	1.7 (15.7%)	bql	43.1 (0.9%)	bql	bql	9.4 (43.9%)	0.3 (46.0%)	17.9 (0.9%)

Table 10. Concentration of metals in mg/L and %RSD in four flavors of Brand B kombucha, five flavors of Brand C kombucha, and brewed teas. *BT stands for black tea, GT stands for green tea, OT stands for oolong tea, HT stands for herbal tea, and K standards for kombucha.

Names	**Al**	**Ca**	**Fe**	**Mg**	**Mn**	**Na**
Ceylon Orange Pekoe^{BT}	2.8 (2.9%)	1.5 (4.6%)	<0.1	5.0 (3.4%)	0.3 (3.1%)	3.0 (2.8%)
Chai French Vanilla^{BT}	3.0 (7.2%)	2.2 (7.5%)	<0.1	4.6 (8.7%)	1.3 (7.9%)	3.7 (6.9%)
Darjeeling^{BT}	2.2 (11.4%)	1.7 (26.1%)	<0.1	6.1 (16.4%)	1.1 (18.7%)	2.7 (5.0%)
Lapsang^{BT}	3.3 (2.2%)	1.8 (10.7%)	bql	4.0 (8.6%)	1.2 (9.3%)	2.6 (2.9%)
Oolong^{OT}	3.3 (3.9%)	2.3 (4.1%)	0.2 (28.6%)	4.7 (3.4%)	2.1 (3.1%)	2.5 (5.7%)
Prince of Whales^{BT}	4.4 (6.2%)	2.1 (12.4%)	0.1 (51.7%)	4.0 (7.9%)	2.0 (7.1%)	2.9 (3.1%)
Green^{GT}	5.1 (3.6%)	2.2 (3.1%)	bql	3.2 (2.6%)	1.5 (2.6%)	3.0 (4.8%)
Yaupon^{HT}	0.9 (7.5%)	1.1 (1.4%)	<0.1	2.9 (5.9%)	0.3 (4.7%)	1.6 (7.9%)
Tony^{HT}	0.8 (8.2%)	0.2 (8.2%)	<0.1	0.2 (19.0%)	<0.1	1.2 (3.9%)
Gyokuro^{GT}	1.2 (3.3%)	0.7 (13.1%)	<0.1	2.0 (2.7%)	1.0 (1.8%)	1.8 (9.0)
BB1a^{K}	2.0 (15.3%)	48.6 (8.5%)	0.1 (5.6%)	22.5 (7.3%)	1.1 (8.3%)	5.7 (7.3%)
BB2a^{K}	1.7 (13.4%)	83.7 (3.0%)	0.2 (63.5%)	42.5 (10.0%)	1.2 (16.9%)	17.9 (6.2%)
BB3a^{K}	1.8 (9.5%)	87.8 (0.9%)	0.3 (6.2%)	45.3 (5.6%)	1.4 (5.2%)	18.6 (6.2%)
BB3b^{K}	1.5 (2.5%)	70.7 (6.6%)	0.2 (4.5%)	35.8 (5.8%)	1.1 (7.3%)	15.7 (4.2%)
BB4a^{K}	1.4 (49.1%)	106.0 (4.0%)	0.4 (43.5%)	49.4 (5.6%)	bql	27.3 (5.2%)
BC1a^{K}	1.8 (6.7%)	16.5 (7.8%)	0.3 (8.5%)	20.1 (12.2%)	1.9 (11.3%)	4.8 (10.4%)
BC2a^{K}	1.9 (4.7%)	2.5 (5.4%)	<0.1	6.5 (4.8%)	2.4 (5.3%)	2.9 (5.7%)
BC3a^{K}	2.2 (6.3%)	27.6 (13.5%)	0.1 (13.3%)	28.1 (12.3%)	2.3 (9.3%)	5.3 (7.9%)
BC4a^{K}	2.5 (14.8%)	39.2 (27.2%)	0.2 (26.1%)	26.5 (26.8%)	2.2 (19.7%)	4.7 (19.3%)
BC5a^{K}	2.6 (16.0%)	6.9 (24.9%)	<0.1	5.5 (24.8%)	1.6 (25.9%)	4.3 (16.7%)

Analyses showed that the concentrations of aluminum, calcium, iron, magnesium, manganese, and sodium were relatively consistent across 10 different Brand A flavors of kombucha. Concentrations in Brand A were similar to concentrations in Brands B and C and brewed tea samples. In general, concentrations of calcium, magnesium, and sodium were higher in Brand A kombuchas, when compared to the concentrations of aluminum, iron, and manganese in those same kombuchas

Concentration Ranges of Metals

ICPOES data for kombucha, brewed tea, and raw tea bags were compiled in Table 11.

Table 11. Concentration ranges of metals in Brands A, B, and C, brewed tea, and raw tea bags. *Sodium emission line not as sensitive at 333.2 nm.

Names	Al	Ca	Fe	Mg	Mn	Na*
Brand A	1.0 -1.5 mg/L	6.0 - 75.0 mg/L	<0.1 - 0.3 mg/L	29.0 - 95.0 mg/L	0.3 – 1.0 mg/L	0.2 - 25.0 mg/L
Brewed Tea	0.8 - 5.0 mg/L	0.2 - 2.0 mg/L	<0.1 – 0.2 mg/L	18.0 - 194.0 mg/L	<0.1 – 2.0 mg/L	1.0 – 3.0 mg/L
Brand B	1.0 – 2.0 mg/L	49.0 - 106.0 mg/L	0.1 – 0.4 mg/L	22.0 – 49.0 mg/L	1.0 – 1.4 mg/L	6.0 - 27.0 mg/L
Brand C	2.0 – 3.0 mg/L	2.0 – 39.0 mg/L	<0.1 – 0.3 mg/L	5.0 – 28.0	1.6 – 2.4 mg/L	3.0 – 5.0 mg/L
Raw Tea Bags	3.0 - 85.0 mg/L	34.0 - 260.0 mg/L	0.5 – 5.0 mg/L	1.4 - 82.0 mg/L	<0.1 - 71.0 mg/L	0.1 – 3.0 mg/L

There were similar concentrations of aluminum, calcium, iron, magnesium, manganese, and sodium in Brands A, B, and C kombuchas. Aluminum concentrations in Brand A ranged between 1.0 mg/L to 1.5 mg/L while the aluminum concentrations in Brand B ranged between 1.0 mg/L to 2.0 mg/L and in Brand C 2.0 mg/L to 3.0 mg/L. Calcium concentrations in Brand A ranged between 6.0 mg/L to 75.0 mg/L while the calcium concentrations in Brand B ranged between 49.0 mg/L to 106.0 mg/L and in Brand C 2.0 mg/L to 39.0 mg/L. Iron concentrations in Brand A ranged between <0.1 mg/L to 0.3 mg/L while the iron concentrations in Brand B ranged between 0.1 mg/L to 0.4 mg/L and in Brand C <0.1 mg/L to 0.3 mg/L. Magnesium concentrations in Brand A ranged between 4.0 mg/L to 32.0 mg/L while the magnesium concentrations in Brand B ranged between 22.0 mg/L to 49.0 mg/L and in Brand C 5.0 mg/L to 28.0 mg/L. Manganese concentrations in Brand A ranged between 0.3 mg/L to 0.8 mg/L while the manganese concentrations in Brand B ranged between 1.0 mg/L to 1.4 mg/L and in Brand C 1.6 mg/L to 2.4 mg/L. Sodium concentrations in Brand A ranged between 0.2 mg/L to 25.0 mg/L while the sodium concentrations in Brand B ranged between 6.0 mg/L to 27.0 mg/L and in Brand C 3.0 mg/L to 5.3 mg/L.

The brewed tea had a higher concentration range of aluminum when compared to Brand A kombucha. The aluminum concentration range was between 0.8 mg/L and 5.0 mg/L in the brewed tea. The concentration ranges of calcium, iron, magnesium, manganese, and sodium in the brewed tea were lower when compared to Brand A. The calcium concentration range was between 0.2mg/L and 2.0 mg/L. The iron concentration ranged from <0.1 mg/L to 0.2 mg/L. The magnesium concentration range was between 0.2 mg/L and 6.0 mg/L. The manganese concentration range was between <0.1 mg/L and 2.0 mg/L. The sodium concentration range was between 1.2 mg/L and 3.7 mg/L. The higher concentration range of aluminum suggest that brewed tea could be responsible for aluminum concentrations in kombucha. The lower concentration ranges of calcium, iron, magnesium, manganese, and sodium suggest that the brewed tea does not contribute these metals to kombucha.

Raw Tea Bags

The raw tea bags were analyzed separately from the brewed tea to determine if the undissolved solids contributed to the concentrations observed in the brewed tea. The concentrations are listed below in Table 12 and Table 13. Silver, beryllium, bismuth, cadmium, cobalt, chromium, vanadium, and zinc were below limit of quantitation. Metals that had concentrations below the instrument's quantitation limit are labeled as (bql). Metals with concentration values less than 0.1 mg/L are listed as <0.1.

Table 12. Concentration of metals in mg/L and %RSD of each raw tea bag. *BT stands for black tea and OT stands for oolong tea.

Metals	**Ceylon Orange Pekoe^{BT}**	**Chai French Vanilla^{BT}**	**Darjeeling^{BT}**	**Lapsang^{BT}**	**Oolong^{OT}**	**Prince of Whales^{BT}**
Al	34.3 (5.4%)	45.3 (2.4%)	28.5 (10.7%)	61.4 (4.4%)	63.2 (8.9%)	77.8 (8.2%)
Ba	0.6 (6.0%)	2.2 (2.0%)	1.3 (11.2%)	2.1 (1.2%)	1.8 (4.1%)	2.4 (4.4%)
Ca	220.2 (11.7%)	213.8 (7.6%)	159.7 (5.7%)	248.0 (2.9%)	214.0 (15.3%)	259.6 (13.7%)
Cu	0.8 (5.4%)	0.4 (2.8%)	0.8 (9.7%)	0.8 (7.8%)	0.7 (50.7%)	0.7 (2.8%)
Fe	1.2 (8.1%)	3.6 (5.1%)	2.6 (16.8%)	4.4 (10.6%)	3.7 (11.1%)	4.6 (8.4%)
Mg	62.0 (9.4%)	75.0 (5.0%)	56.5 (6.6%)	82.2 (7.6%)	67.5 (8.3%)	81.8 (4.9%)
Mn	5.9 (6.2%)	43.9 (4.2%)	17.1 (1.9%)	42.6 (3.9%)	49.7 (9.1%)	71.0 (7.4%)
Na	1.6 (10.8%)	2.6 (3.6%)	1.3 (21.6%)	2.0 (21.6%)	2.0 (10.2%)	2.4 (6.5%)
Ni	<0.1	<0.1	<0.1	0.1 (11.9%)	<0.1	<0.1
Pb	<0.1	<0.1	<0.1	<0.1	0.1 (8.4%)	0.1 (82%)

Table 13. Concentration of metals in mg/L and %RSD of each raw tea bag. *BT stands for black tea, GT stands for green tea, and HT stands for herbal tea.

Metals	Green^{GT}	Yaupon^{HT}	Humi^{BT}	Tony^{HT}	Gyokuro^{GT}
Al	85.1 (7.6%)	3.5 (3.3%)	6.8 (2.5%)	24.3 (61.2%)	5.2 (5.3%)
Ba	2.7 (3.6%)	0.7 (3.4%)	0.5 (2.2%)	0.8 (6.3%)	0.1 (9.1%)
Ca	248.0 (16.1%)	50.0 (8.7%)	34.0 (2.9%)	38.3 (1.5%)	57.9 (3.8%)
Cu	66.0 (5.8%)	<0.1	0.2 (7.0%)	0.1 (14.8%)	0.1 (15.7%)
Fe	68.7 (6.9%)	0.5 (2.2%)	0.8 (3.1%)	0.7 (5.5%)	1.0 (2.5%)
Mg	1.4 (9.0%)	72.7 (1.6%)	29.7 (5.6%)	38.9 (17.0%)	14.5 (4.8%)
Mn	<0.1	13.8 (2.3%)	6.1 (0.8%)	26.0 (54.5%)	10.4 (2.9%)
Na	0.1 (13.7%)	0.8 (2.7%)	0.3 (15.2%)	0.7 (99.1)	2.6 (26.9%)
Ni	1.1 (4.1%)	bql	bql	bql	bql
Pb	bql	bql	bql	bql	bql

The raw tea bags had higher concentrations of aluminum, calcium, magnesium, iron, and manganese when compared to the brewed tea concentrations in Table 10 and concentration ranges in Table 11. This is likely due to the acid digestion of the undissolved solids after brewing the tea. The aluminum concentration range was between 3.5 mg/L and 85.1 mg/L. The calcium concentration range was between 34.0 mg/L and 260.0 mg/L. The iron concentration range was between 0.5 mg/L and 4.6 mg/L. The magnesium concentration range was between 1.4 mg/L and 82.0 mg/L. The manganese concentration range was between <0.1 mg/L and 71.0 mg/L. The higher concentration ranges of aluminum, calcium, magnesium, iron, and manganese suggest that the undissolved solids are not responsible for the metal concentrations observed in the brewed tea.

ICPOES Analysis of Water

Brand A's source water and WCU's Natural Science Room 217 ultrapure water used in all dilutions and standards were analyzed to determine if they were sources of metals that were observed in the Brand A kombucha. Table 14 shows the concentrations in both waters. Silver, barium, beryllium, bismuth, cadmium, cobalt, chromium, copper, manganese, nickel, lead, vanadium, and zinc were below limit of quantitation. Metals that had concentrations below the instrument's quantitation limit are labeled as (bql). Metals with concentration values less than 0.1 mg/L are listed as <0.1.

Table 14. Concentration of metals in mg/L and %RSD of Brand A and WCU Natural Science Room 217 ultrapure water.

Samples	Al	Ca	Fe	Mg	Na
Brand A Water	bql	20.7 (32.7%)	<0.1	2.2 (26.2%)	2.5 (20.6%)
WCU NS 217 Ultrapure Water	0.6 (13.9%)	bql	<0.1	<0.1	2.1 (7.2%)

The calcium concentration in Brand A's water was 20.7 mg/L, which falls within the calcium concentration range of Brand A kombucha in Table 11. This suggests that Brand A water may be responsible for the calcium concentrations observed in Brand A. The iron concentration is <0.1 mg/L which is seen in Brand A in Table 9. This suggests that Brand A water may be contributing iron to Brand A. Magnesium and sodium concentrations in the water fell below the concentration ranges observed in Brand A in Table 11. This suggests that magnesium and sodium are not responsible for the concentrations observed in Brand A. WCU's Natural Science Room 217 ultrapure water had low concentrations of aluminum, magnesium, and sodium when compared to Brand A. The aluminum concentration was 0.6 mg/L, magnesium concentration was <0.1 mg/L, and the sodium concentration was 2.1 mg/L. This suggests that aluminum, magnesium, and sodium in WCU's Natural Science Room ultrapure water are not responsible for the concentrations observed in Brand A. The iron concentration was <0.1 mg/L which is seen in Brand A in Table 9. This suggests that the iron in WCU's water may be contributing to the iron concentrations in Brand A kombuchas.

ICPOES Analysis of Source Material

Sugar, Humi Tea, Fermented Tea A, Fermented Tea B, Kombucha Al, and Base Sweet Tea concentrations are presented in Table 15. Silver, barium, beryllium, bismuth, cadmium, cobalt, chromium, copper, nickel, lead, vanadium, and zinc were below limit of quantitation. Metals that had concentrations below the instrument's quantitation level are labeled as (bql). Metals with concentration values less than 0.1 mg/L are listed as <0.1.

Table 15. Concentration of metals in mg/L and %RSD of Brand A's source materials. *SM stands for source material.

Samples	Al	Ca	Fe	Mg	Mn	Na
Sugar^{SM}	0.6 (1.8%)	0.2 (0.0%)	Bql	<0.1	bql	1.1 (1.4%)
Humi Tea^{SM}	1.2 (1.3%)	0.3 (6.2%)	<0.1	1.5 (7.6%)	0.2 (6.5%)	1.2 (5.5%)
Fermented Tea A^{RM}	1.0 (6.7%)	26.2 (1.4%)	<0.1	4.9 (2.4%)	0.3 (4.5%)	4.2 (2.0%)
Fermentd Tea B^{SM}	0.3 (9.1%)	47.2 (3.9%)	0.3 (2.9%)	6.5 (1.1%)	0.6 (2.6%)	4.8 (0.4%)
Kombucha Al^{SM}	10.1 (1.7%)	6.1 (7.1%)	0.1 (15.7%)	8.9 (1.8%)	4.8 (4.9%)	6.1 (1.3%)
Base Sweet Tea^{SM}	bql	35.4 (5.5%)	<0.1	4.3 (0.7%)	0.3 (1.8%)	4.2 (3.0%)

Sugar had low concentrations of aluminum, calcium, magnesium, and sodium when compared to Brand A kombuchas in Table 9. The aluminum concentration was 0.6 mg/L, the calcium concentration was 0.2 mg/L, the magnesium concentration was <0.1 mg/L, and the sodium concentration was 1.1 mg/L. The Humi tea had low concentrations of calcium, magnesium, manganese, and sodium when compared to the concentration ranges in Brand A. The concentration of iron was <0.1 mg/L which is similar to the concentrations observed in the Brand A and the company water. Humi tea had similar concentrations of aluminum, when compared to Brand A in Table 9. The Humi tea aluminum concentration was 1.2 mg/L, which falls within the aluminum concentration range in Table 11.

The concentrations of aluminum in Fermented Tea A was 1.0 mg/L, Fermented Tea B was 0.3 mg/L, and kombucha al was 10.1 mg/L. Kombucha Al had a higher aluminum concentration than Brands A, B, and C kombuchas. Fermented Tea A aluminum concentration was within range of Brands A, B, and C. Fermented Tea B aluminum concentration was below the concentration range of Brands A, B, and C. The concentrations of calcium in Fermented Tea A was 26.2 mg/L, Fermented Tea B was 47.2 mg/L, Kombucha Al was 6.1 mg/L, and Base Sweet Tea was 35.4 mg/L. These concentrations were within the concentration ranges of Brands A and C. The concentrations of iron in fermented tea A was <0.1 mg/L, Fermented Tea B was 0.3 mg/L, Kombucha Al was 0.1 mg/L, and Base Sweet Tea was <0.1 mg/L. These concentrations were within the concentration ranges of Brands A, B, and C. The concentrations of magnesium in Fermented Tea A was 4.9 mg/L, Fermented Tea B was 6.5 mg/L, Kombucha Al was 8.9 mg/L, and Base Sweet Tea was 4.3 mg/L. These concentrations were below the concentration ranges of Brands A and B. The concentrations of manganese in Fermented Tea A was 0.3 mg/L, Fermented Tea B was 0.6 mg/L, Kombucha Al was 4.8 mg/L, and Base Sweet Tea was 0.3 mg/L. These concentrations were within the concentration ranges of Brand A. The concentrations of sodium in Fermented Tea A was 4.2 mg/L, Fermented Tea B was 4.8 mg/L, Kombucha Al was 6.1 mg/L, and Base Sweet Tea was 4.2 mg/L. These concentrations were within the concentration ranges of Brands A and C.

CHAPTER SIX: CONCLUSION

The purpose of this thesis project was to develop and perform kombucha quality analysis methods for a regional company. The project consisted of determining compounds of interest in finished kombucha and source materials. This would help the company to gauge product quality and help with identifying new source materials. Sample treatment assays and instrument methods were developed to test for each family of compounds. IC was used to determine anions while ICPOES was used to determine metals. IC analysis involved a determination of eight anions while ICPOES involved a multi-elemental determination of 18 metals.

Anion concentrations of acetate, chloride, nitrate, phosphate, and sulfate varied between each Brand A kombucha flavor. This is likely due to the different additives, such as; fruit juice, spices, and extracts. Acetate was found in the highest concentration, which is to be expected because acetic acid is a major by-product of sweet-tea fermentation. Analyses showed that the concentrations of aluminum, calcium, iron, magnesium, manganese, and sodium were relatively consistent across the 10 different kombucha flavors of Brand A, and were similar to concentrations in Brands B and C. In general, calcium, magnesium, and sodium were the most prevalent metals found in the 10 Brand A kombucha flavors.

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