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Antioxidant Potentials of Kombucha Based on Water Chemistry and its Impact on Fermentation

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Abstract: Kombucha is made by using a symbiotic culture of bacteria and yeast (SCOBY) to ferment sweetened tea. This fermentation produces a beverage with a unique aroma and acidic flavor. Kombucha has recently gain popularity in the United States and has been reported to have numerous health benefits from increased weight loss to helping to fight cancer. There is a wide variation in kombucha composition, little is known about the impact the water's chemistry has on the fermentation and the resulting kombucha. The impact that bicarbonate, chloride, calcium, magnesium, and sulfate concentration have on the measured antioxidation potential of brewed kombucha was investigated. Effects were investigated both for individual water ionic compositions. This information can be used to help produce kombucha with an optimized chemical profile and improved antioxidant potentials.

Introduction

Kombucha is a fermented tea beverage known for its far-reaching health benefits that has become rapidly popularized. Kombucha originated in China over 2000 years ago where it was suspected of having magical properties prized for having energizing and detoxifying effects (Greenwalt et al., 2000). It spread to neighboring countries and then made its way to Eastern Europe at the beginning of the 20th century (Dufresne & Farnworth, n.d.). Today kombucha is distinguishable by its distinct flavor, mildly acidic and alcoholic, with a similar taste to apple cider vinegar (Jarrell et al., 2000). Kombucha is prepared by using black, green, or white tea, sugar, and adding a symbiotic culture of acetic acid bacteria and yeast (SCOBY). These microorganisms can be found in a microbial colony floating on top of the beverage, this known as a pellicle (Greenwalt et al., 2000). The sugar addition is required for the microorganisms to grow and the beverage to ferment. Fermentation takes place during an approximately 1–2 week timeframe. There are different aspects of the brewing and fermentation process that will change the different properties of Kombucha. These could include the fermentation time, temperature, changes in microbial composition, and ion concentration of the water for brewing (Watawana et al., 2015).

One reason Kombucha has been rapidly becoming more popular is the large number of claimed health benefits. Common health issues such as headaches, arthritis, diabetes, fatigue, old age, cancer, and cardiovascular disease have been documented to be cured by consuming Kombucha daily (Greenwalt et al., 2000). Kombucha is known to balance the microbial composition of the gastrointestinal tract, acting as a probiotic drink. The low pH and the acetic acid content of Kombucha accounts for the antibacterial and antifungal properties observed (Watawana et al., 2015). While these effects have been well documented, there has been limited pharmacologically focused research into kombuchas effectiveness. However, with the increased interest in kombucha, it is anticipated that there will be a correlated increase in the amount of research to support the marketed claims.

One beneficial characteristic of Kombucha is that it is known for having a high concentration of antioxidants that helps the body maintain redox homeostasis and enhances its immunity (Jakubczyk et al., 2020) Antioxidants, nicknamed “free radical scavengers”, inhibit the oxidation process, and balance out the free radicals generated during metabolic processes (A Review on Free Radicals and Antioxidants, 2018). Free radicals are molecules containing oxygen that have an uneven or unpaired number of electrons. Due to this, they react with stable biological molecules to stabilize themselves (Carocho et al., 2018). Overproduction of these molecules disturbs redox homeostasis and can cause numerous diseases such as cancer, atherosclerosis, and neurological disorders (Yi et al., 2021). The body produces antioxidants endogenously, but they can only balance a limited number of free radicals. So, it is important that the body is also supplied antioxidants exogenously through fruits, vegetables, and different types of beverages such as Kombucha. By electron transfer and its hydrogen donating ability, kombucha can neutralize these radials and improve overall health (Lobo et al., 2017).

The impact of the total fermentation time of black tea Kombucha on the free-radical scavenging ability, total phenolic compounds, and titratable acidity has been reported (Ahmed et al., 2020). It was found using the oxidation of 2,2′-Azino-bis-(-3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) that the antioxidant activity increased with fermentation time, the phenol compounds were found to increase using the Folin-Ciocalteau method, and the titratable acidity increased (Srihari & Satyanarayana, n.d.) An increase of the consumption of Kombucha with its high antioxidant and phenolic composition can protect the body from diseases caused by free radicals and therefore contribute to the health benefits. It has also been reported that an increase in the concentration of sugar results in an increase in the number of metabolites (Greenwalt et al., 2000). Jabubczyk et al. (2020) investigated the effect of the tea type used (black, green, white, and red tea) on the chemical potential of the Kombucha Once fermented, kombucha samples were tested for antioxidant potential, phenols, flavonoids, pH, alcohol, and sugar content (Jakubczyk et al., 2020). It was found green tea had the highest antioxidant potential and phenol content while black tea had the lowest. After the 14^{th} day of fermentation, the chemical components began to decline, indicating a limit for fermentation time (Jakubczyk et al., 2020). Of the current kombucha research reported, one aspect that hasn't been researched is the component of brewing that is the most abundant, water.

Water is one of the most important factors to consider when brewing tea and kombucha because it needs to be free of any contaminants that could harm the microorganisms in the SCOBY (Wilson, 2008). The mineral content can also impact the final flavor and chemical composition of the beverage due to how the microorganisms react and use them. There are different types of water that are available to use, including well water, spring water, bottled water, and municipal tap water (Crum et al., 2016). The different water sources will have different mineral content, which will impact the product in numerous ways. In tap or public water sources they usually contain a significant amount of chlorine, which is one mineral that would impact the culture in a negative way due to its antibacterial nature and can make the final product have a detergent-like taste (Fermentation, n.d;) (Crum et al., 2016). While the impact of water chemistry on the brewing of Kombucha is not well studied, it has a known influence on the brewing of other beverages such as beer and whisky (Fermentation, n.d.). For beer, the major brewing companies have ionic profiles for the water they use during their brewing process to control the sensory aspects of their product. These profiles include a list of ions and their concentrations to keep the brewing and taste of their products optimized and consistent (Fermentation, n.d.). This same approach could prove to be very impactful on the brewing of Kombucha.

Aiming to optimize the positive attributes of fermented Kombucha for homebrewers and commercial brewers, the effects of ionic chemistry when brewing the black tea and fermenting Kombucha were investigated. These ions include bicarbonate, chloride, calcium, magnesium, and sulfate. To test the differences in chemical composition between the different ions the % scavenging effect of antioxidants with DPPH were evaluated. These characteristics were analyzed pre- and post-fermentation. The results were compiled and then relations between the different ions and their concentrations were analyzed to draw conclusions.

Materials and Methods

2.1 Kombucha Preparation and Fermentation

A black tea blend and kombucha starter kit was purchased from Northern Brewer located in Roseville, Mn. The kombucha starter, known as SCOBY, was packaged by Oregon Kombucha located in Vancuver, Wa. Reagent alcohol (95%), sodium bicarbonate, and sodium chloride were purchased from VWR International located in Radnor, Pa. Sodium sulfate and calcium nitrate tetrahydrate were purchased from EM Science located in Gibbstown, NJ an affiliate of Merck KGaA located in Darmstadt, Germany. Magnesium nitrate was purchased from Mallinckrodt Baker, Inc. located in Phillipsburg, NJ. All chemicals were used without further modification or purification. All solutions were prepared using class A volumetric glassware and an analytical balance (±0.0001g).

To prepare the base black tea, 1000mL of DI water was brought to a boil and then 100g of raw sugar cane was completely dissolved. For the specific ionic concentrations being tested, the calculated grams of the ion corresponding with the desired concentration was added and dissolved before adding the raw cane sugar. Then 8g of the black tea blend was added and steeped for 15 minutes. It was then filtered with a Buchner funnel and cooled in the refrigerator for approximately 2 hours. To prepare the kombucha, 255mL of the cooled tea was transferred to three separate flasks and 45mL of *SCOBY* with a small piece of pecil was added to each flask. These were labeled accordingly and stored at 21°C for approximately 14 days. The finished kombuchas were transferred into storage containers and labeled and stored at 4 °C until testing.

2.2 Antioxidant Activity of Kombucha

Antioxidant activity was measured using 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl (DPPH), 97% purchased from Matrix Scientific Located in Richland County, SC. The spectral absorbances were measured at 517nm using a Shimadzu Scientific UV-1800 Spectrophotometer (Kyoto, Japan).

A high free radical solution was made with 100mL of 95% reagent alcohol $9.89*10^{-5}$M DPPH. 3mL of this solution was combined with 1mL of a selected tea or kombucha sample, shaken, and incubated in the dark for 30 minutes to allow for the reaction to take place. Each sample, tea or kombucha, was evaluated five times. This testing scheme is shown in figure 1 for the kombucha samples. After the incubation time was up, the absorbance of the DPPH solution before and then after the addition of the sample of interest was measured at 517nm. These measurements were then used to calculate the scavenging effect of the sample following the equation below.

$$\text{Scavenging Effect (\%)} = \frac{A_0 - A_S}{A_0}$$

A_0 is the absorbance at 517nm of the high free radical solution of DPPH

A_S is the absorbance at 517nm of the DPPH solution and the sample of interest.

2.3 Commercially Produced Kombucha Products

Six commercially produced kombuchas were purchased from local grocery stores or directly from local producers. These kombuchas are coded as CK1 – CK6. All samples were stored in original packaging and stored at 4 °C until testing.

Results and Discussion

To optimize the positive chemical attributes of kombucha, the effects of changing ionic water chemistry were investigated. The antioxidant potentials of the different samples of kombucha were assessed using a free radical DPPH before and after fermentation. The results were compiled and graphed to analyze the relationships between ion and concentration.

3.1 Commercially Purchased Kombucha Products

The first kombucha samples that were assessed were commercially purchased brands of Kombucha. The antioxidant potentials of each brand were calculated, these values were compiled to set a standard for the kombucha samples to be tested. These values represent the average of all the absorbance values measured at 517nm for each sample. The standard deviations for these averages were calculated and shown as well. These values are shown in figure 2. The antioxidant potentials of these kombucha brands were calculated to range from 36.98 (±3.50) % to 95.13 (±0.79) %. Overall, these values showed a high percentage and indicate that the kombucha beverages that are sold commercially have a high number of antioxidants that can have positive health benefits. When comparing these values to the literature value of black tea kombucha of 61.04 (±1.99) %, the scavenging effects for the commercial products lay significantly higher than the literature value except for CK4 which was significantly lower (Jakubczyk et al., 2020). Then, the first experimental kombucha sample was prepared using deionized (DI) water. The antioxidant potential before fermentation, as a tea product, was calculated to be 86.26 (±1.25) %. Then it was measured again after fermentation, as a kombucha product, to be 85.69 (±2.46) %. Considering the standard deviations in these values, the antioxidant potential for the DI tea and kombucha was the same. So, there was no different pre- or post-fermentation on this potential. In comparison with the literature value, the DI water tea and kombucha were already at a significantly higher scavenging effect. In comparison with the commercial brands of kombucha, the DI water fit the range of antioxidant potentials that were calculated. This sample is the starting point to see how the antioxidant potential will change for the tea and kombucha samples as different ions of different concentrations are added.

3.2 Bicarbonate (HCO_3^-)

The antioxidant potential of kombucha when adding a high, medium, and low concentration of bicarbonate was calculated and these are shown in figure 3. The high concentration of 200mg/L had a tea antioxidant potential of 55.14 (±0.88) % and for the kombucha it was 78.21 (±4.01) %. For the medium concentration of 100mg/L the potential of the tea was 68.18 (±0.46) % and the kombucha was 84.12 (±2.84) %. The potential for the low concentration of 50mg/L was 81.42 (±0.96) % for the tea and 78.65 (±0.80) % for the kombucha.

The scavenging effects observed in the kombucha fermented with the bicarbonate were below the scavenging effects observed from kombucha fermented with DI water. It was within the range observed in the commercial kombucha products and higher than the value shown in literature. These results show a dramatic increase in the scavenging effect for the tea samples, with an increase of almost 30%. For the kombucha, when considering the standard deviations for the three concentrations, there was no observable increase or decrease in the final scavenging effect. When comparing the tea samples to the kombucha samples of the same concentration, a significant increase was seen for the high and medium concentrations. However, for the low concentration, the scavenging effect of the tea surpassed that of the kombucha. From this it can be stated that decreasing the concentration of the bicarbonate has an inverse relationship with the scavenging effect of the tea. On the other hand, fermentation will keep the scavenging effects in the kombucha consistent despite the change in concentration.

3.3 Chloride (Cl^-)

For the chloride ion the antioxidant potentials calculated are shown in figure 4. For the high concentration of 150mg/L the potential of the tea was 86.82 (±0.98) % and the kombucha was 88.21 (±2.53) %. The medium concentration of 75mg/L had a tea potential of 80.59 (±1.21) % and a kombucha potential of 82.77 (±1.13) %. For the low concentration of 25mg/L the calculated potential for the tea was 83.84 (±1.11) % and for the kombucha was 86.07 (±2.08) %. From this data, the values calculated stayed consistent despite the changes in the concentration of chloride. The tea and kombucha samples had similar values, indicating that fermentation did not have a significant effect on the scavenging effects of the samples. The scavenging effects observed when fermenting with chloride ions are slightly increased from those observed from the kombucha fermented with DI water. It lays in the higher range of the values observed in the commercial products. Overall, the percentages are high so while changing the concentration of calcium does not have an observable impact, it does interact positively with the brewing and fermenting process to produce a product with a high antioxidant potential.

3.4 Calcium (Ca^{2+})

The tea and kombucha antioxidant potentials calculated when adding the high, medium, and low concentrations of calcium are shown in figure 5. For the high calcium concentration of 100mg/L, 89.70 (±0.79) % was the potential calculated for the tea and 86.06 (±2.78) % for the kombucha. The medium concentration of 50mg/L had a tea potential of 83.30 (±0.67) % and a kombucha potential of 85.45 (±1.17) %. The potentials of the low concentration of 25mg/L for the tea was 86.55 (±1.84) % and for the kombucha was 81.73 (±2.28) %. The scavenging effect for the DI water kombucha was in the same range as the samples fermented with calcium for the higher concentrations. The lower concentration of calcium had a scavenging effect below the DI sample value. Overall, for the tea samples, there was a decrease in the percentage for the medium concentration while the high and medium had around the same percentage. The kombucha samples had a correlated decrease in scavenging effect with the decrease in concentration. Considering the standard deviations of these values, the decrease is less significant. For the overall data of the tea and kombucha it can be determined that changing the calcium concentration has a slight relational impact on the scavenging effects of the tea and kombucha samples.

3.5 Magnesium (Mg^{2+})

Figure 6 shows the antioxidant potentials for the high, medium, and low concentrations of the samples prepared with magnesium. For the high concentration of 100mg/L the tea was calculated to be 90.63 (±2.05) % and 85.97 (±3.01) % for the kombucha. The medium concentration of 50mg/L had a tea potential of 89.22 (±0.54) % and 86.82 (±1.60) % for the kombucha. For the low concentration of 25mg/L the tea was calculated to be 87.65 (±0.54) % and was 81.21 (±3.25) % for the kombucha. From this data there is a decrease in the tea scavenging effect with the decrease in magnesium concentration. For tea, these values start in the nineties and end in the high eighties, which are the highest potentials that have been seen thus far. Comparing the kombucha values with that of the DI kombucha, they fall within the same range when considering the standard deviations. The magnesium kombucha samples fall in the middle of the scavenging effects for those purchased commercially. The tea and the kombucha follow the same general pattern as from the high to the low concentration there is a decrease in potential, while the medium concentration has the highest. Overall, there is an observable decrease in the scavenging effects for both the tea and kombucha samples with a decrease in the magnesium concentration.

3.6 Sulfate (SO_4^{2-})

The antioxidant potentials for the samples made with sulfate were calculated for the high, medium, and low concentrations and shown in figure 7. The high concentration was 400mg/L and the potential for the tea was 81.67 (±0.62) %, while for the kombucha it was 83.50 (±3.90)%. The medium concentration of 200mg/L had a tea potential of 71.53 (±2.18) % and the kombucha sample was 85.17 (±2.15) %. The low concentration of 100mg/L was 89.19 (±0.87) % for the tea and 85.94 (±5.40) % for the kombucha. This data shows that for the tea samples, decreasing the concentration led to a slight increase in the scavenging effect. The medium concentration did have a surprisingly low value between the high and low concentrations. Considering the standard deviations, there was an observable increase for the potential of the brewed tea samples. For the kombucha samples, there was an observed increase in the average scavenging effects with the decrease in concentration. However, the scavenging effects did not have a significant change when taking into consideration the standard deviations of the values. So, for sulfate it can be determined that decreasing the concentration has a positive effect on the potential of the tea samples but regarding the kombucha samples there was no such effect. The scavenging effects observed in the kombucha samples fermented with sulfate were within the same range of those observed from the sample fermented with just DI water. Considering the standard deviation for the low concentration of sulfate, the range does expand past the DI samples but on average they were the around the same. The sulfate samples have values that reside in the middle of the scavenging effects of the commercial products. So, overall, there wasn't much of a positive or negative effect for the scavenging effects of the kombucha samples fermented with sulfate.

Conclusion

The effects on the final antioxidant potential of black tea and fermented Kombucha were investigated based on changing the ionic chemistry of the water used. The data was analyzed to determine what concentrations of what ions resulted in the highest scavenging effect to optimize the health benefits and other positive attributes of the beverages. Out of the five ions: bicarbonate, chloride, calcium, magnesium, and sulfate it was observed that the high concentration of chloride had the highest scavenging effect for kombucha of 88.21 (±2.53) %. For the tea, the highest concentration of magnesium had the highest scavenging effect of 90.63 (±2.05) %. Overall, the average of all the scavenging effects for the tea was 89.61% and for the kombucha it was 83.99%. This shows that the kombucha samples did have slightly higher antioxidant potentials on average than the non-fermented black tea samples. When comparing the scavenging effects of kombucha samples with differing ionic concentrations to the literature value, all of the samples had scavenging effects that were significantly higher. Comparing them to the standard DI fermented kombucha, there were no samples that stood out to be significantly higher or lower. Overall, the results did show high values of antioxidant potentials and proved that the claimed health benefits of kombucha products do correlate.

Going forward, a high, medium, and low concentration composite will be made and tested for tea and kombucha. These composites will contain all five of the ions and their concentrations previously tested. Expanding upon testing the antioxidant potentials, pH, titratable acidity, ethanol, and free amino nitrogen (FAN) will also be tested for each sample to gather a full chemical profile. Once all the data is collected and compiled together, statistical analyses will be performed to determine differences and similarities between the ions and their concentrations.

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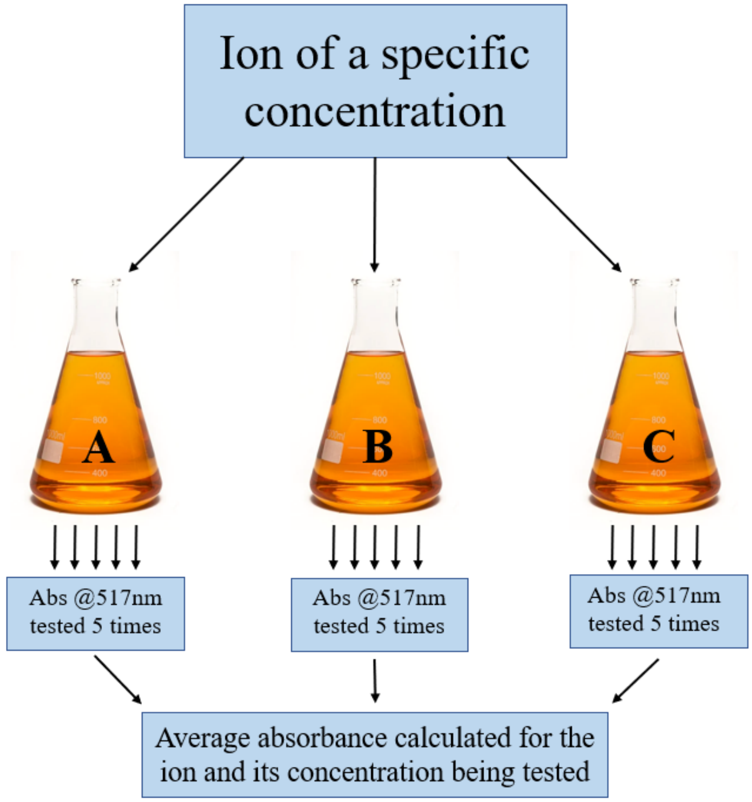

Figure 1: The figure above shows the scheme for testing the kombucha samples. Each ion of a specific concentration was made into three kombucha samples for accuracy. Then the scavenging effect of each kombucha sample was tested 5 times. The average of these fifteen scavenging effects was calculated to obtain one value for the ion and its concentration being tested.

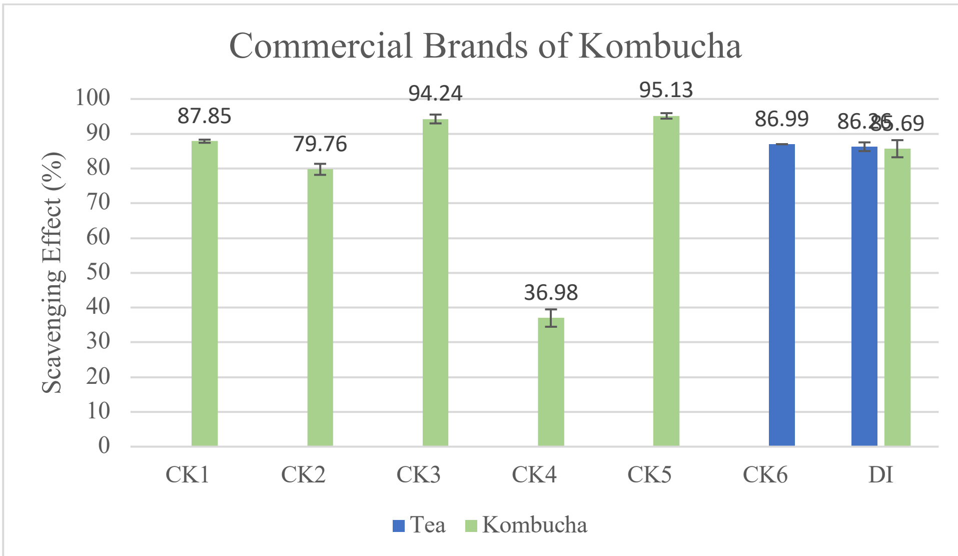

Figure 2: The figure above shows the scavenging effects (%) for 7 commercially purchased brands of Kombucha. It also shows the first batch of experimentally made tea and fermented Kombucha that was prepared with deionized (DI) water. Above each bar is the samples individually calculated SE value (%).

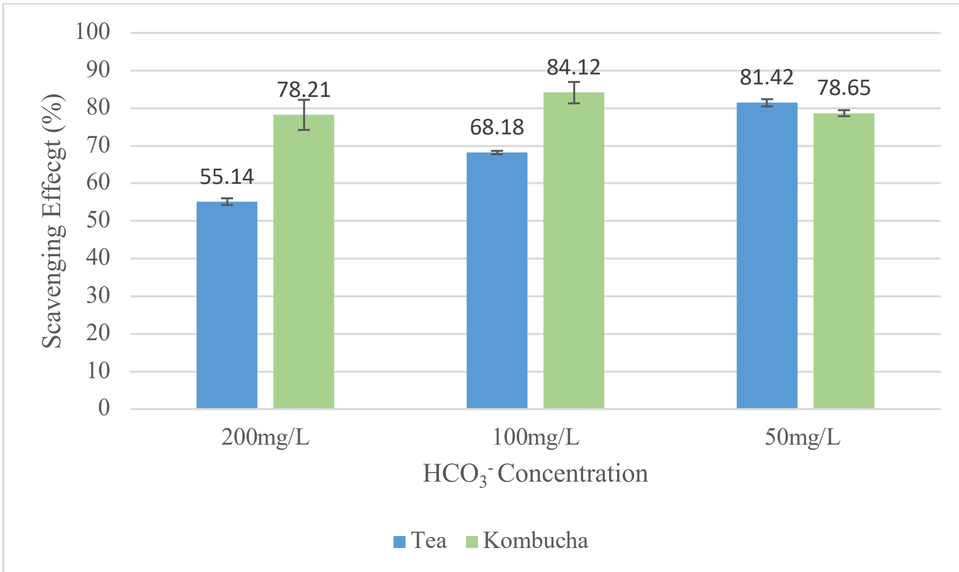

Figure 3: This figure shows the scavenging effects (%) of the samples prepared with bicarbonate (HCO_3^-). From left to right is the high to low concentrations of bicarbonate, being 200mg/L, 100mg/L, and 50mg/L. Each concentration is shown with two bars, a tea (blue) and kombucha (green) sample shown next to each other for comparison.

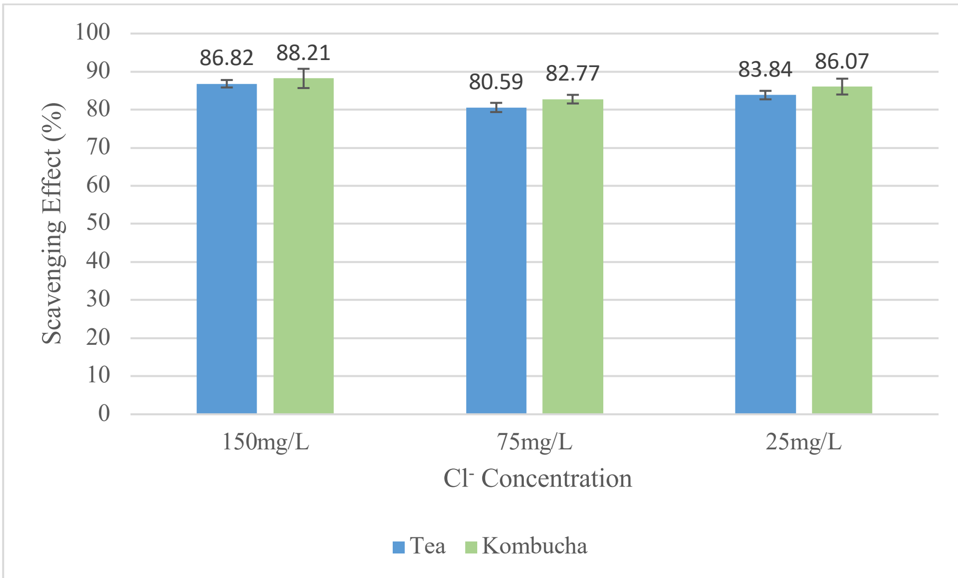

Figure 4: The figure above shows the scavenging effects (%) of the samples prepared with chloride (Cl^{-}). From high to low the concentrations made were 150mg/L, 75mg/L, and 25mg/L. Each concentration has the tea (blue) and kombucha (green) samples measured and their values above the bars.

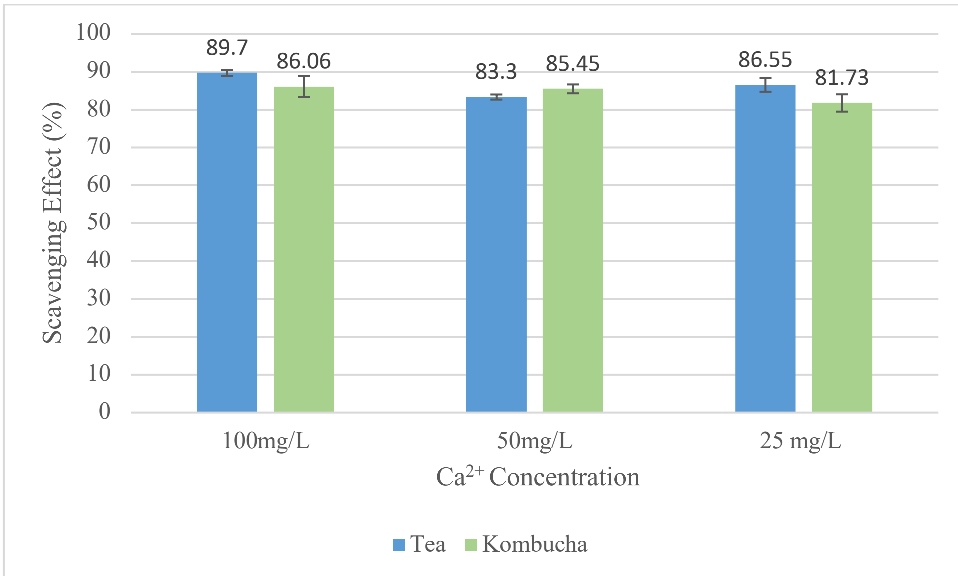

Figure 5: The figure above shows the scavenging effects (%) for the samples prepared with calcium (Ca^{2+}). From left to right the concentrations made were 100mg/L, 50mg/L, and 25mg/L. The tea (blue) and Kombucha (green) samples are shown next to each other with their individual values above their bars.

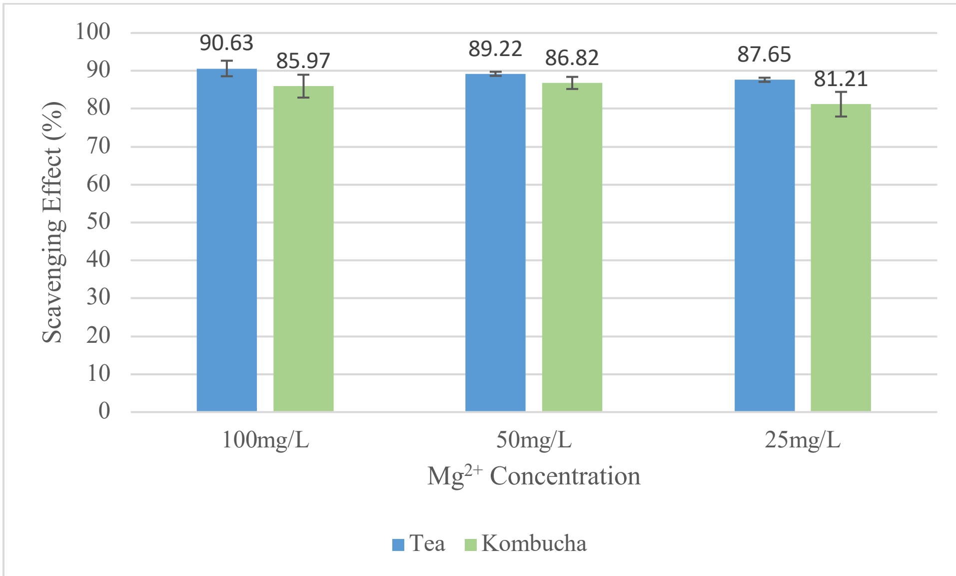

Figure 6: The figure above shows the scavenging effects (%) for the samples prepared with magnesium (Mg^{2+}). From left to right the concentrations made were 100mg/L, 50mg/L, and 25mg/L. The tea (blue) and Kombucha (green) samples are shown next to each other with their individual values above their bars.

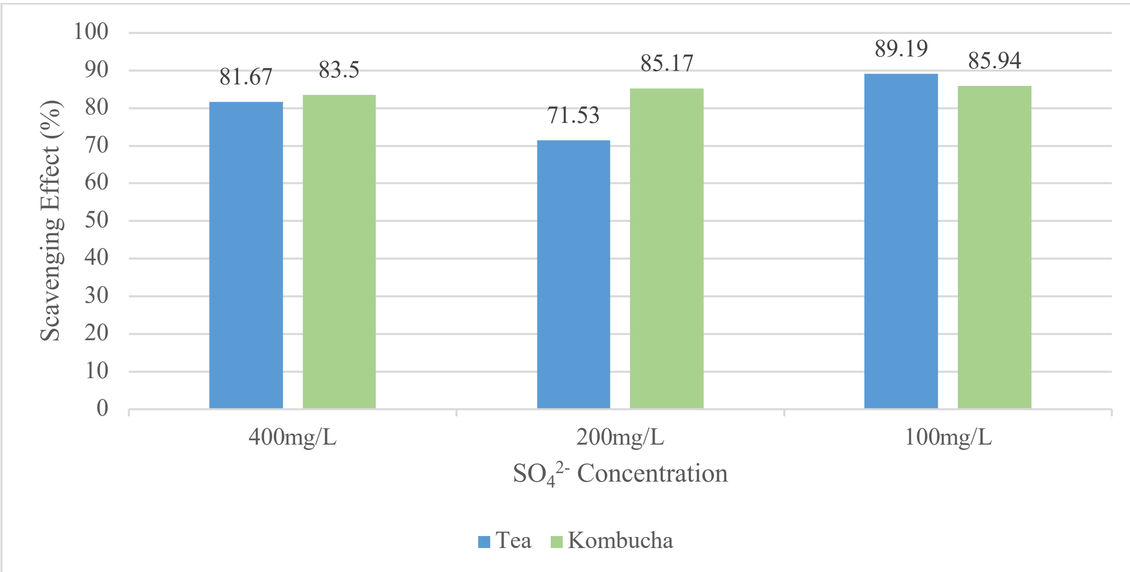

Figure 7: Shown in the figure above is the scavenging effects (%) for the samples prepared using sulfate (${SO_4}^{2-}$). On the left is the high concentration sample of 400mg/L, then 200mg/L and then 100mg/L. The tea (blue) and Kombucha (green) samples are shown next to each other with their individual values above their bars.