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Characterization of Active Compounds Produced in the Biotransformation of Metabolites in Kombucha Tea

Katherine Seehusen
Winona State University

Mallory Villeneuve
Winona State University

Francis Mann
Winona State University

CHARACTERIZATION OF ACTIVE COMPOUNDS PRODUCED IN THE BIOTRANSFORMATION OF METABOLITES IN KOMBUCHA TEA

Katherine J. Seehusen, Mallory A. Villeneuve, Francis M. Mann
Department of Chemistry, Winona State University, Winona, MN 55987

ABSTRACT

Kombucha is a fermented black tea that has been hypothesized to provide many health benefits. The exact origin of these benefits, however, is continually being investigated. This study aims to identify the various active compound produced in the biotransformation of the metabolites during the fermentation process of the tea beverage, as well as quantify functions such as antioxidant capacity. Hydrophilic and hydrophobic liquid-liquid extractions were performed on a filtered sample of GT's Organic Raw Kombucha® using acetonitrile and ethyl acetate. Ferric reducing/antioxidant power (FRAP) assays were run on both the Kombucha tea and the extracts to determine their antioxidant capacity. The Kombucha tea, acetonitrile extract, and ethyl acetate extract were found to have FRAP values of 146.9, 102.7, and 71.04, respectively. The high retention of FRAP in the acetonitrile extract gives evidence that a polar hydrophobic molecule is functioning as an antioxidant in the Kombucha tea. Antioxidant capacity was further tracked using a variety of chromatographic techniques including the use of silica gel and C-18 functionalized silica flash chromatographies, as well as reverse-phase C-18 HPLC. These studies aim to further purify and characterize the compounds responsible for antioxidant capacity.

BACKGROUND

Kombucha has long been perceived to possess compounds that provide beneficial health effects; however, the exact origin of theses effects remains unknown. Kombucha, a fermented tea, is traditionally prepared using black tea and sucrose. Glucose and fructose, hydrolyzed from sucrose, are used by the symbiotic culture of bacteria and yeast during the aerobic conditioned incubation period usually lasting 7-10 days.[1] Kombucha contains many metabolites, amino acids, proteins, and other substances such as phenolics, which are believed to contribute heavily to the health benefits. Some supposed sources for the health benefits include the microbial effects and antioxidant activities of both the tea and Kombucha. Kombucha appears to possess antimicrobial properties, even from compounds in the Kombucha other than acetic acid and large proteins.[2] Many humans have claimed to see positive health results from regular Kombucha consumption including improvement of arthritis, cancer, and an increased immune response. The health benefits have been studied in rats, which after ingesting Kombucha showed increased longevity and alertness. Studies have been completed to analyze the fermentation process and determine the specific role of the fermentation process in the antioxidant capacity of Kombucha. By using radical scavenging assays, it was shown that the radical scavenging activities increased as a function of time in the fermentation process. In addition, the total phenolic content was seen to increase during fermentation.[3] It has been previously found that phenolics compose about one third the dry mass of tea.[4] Flavonoids are the most abundant of this class, including catechins.[1] This study aims to characterize the molecules responsible for antioxidant activity in Kombucha in comparison to black tea.

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Department of Chemistry

EXPERIMENTAL APPROACH

Liquid-liquid extractions were first performed on Kombucha and BT using acetonitrile and ethyl acetate to separate the components based on hydrophobic/hydrophilic and polarity properties, and acid hydrolysis was performed using 10% MeOH/5 M HCl to cleave glycosidic bonds.[5] The antioxidant capacities of the samples were analyzed via Ferric Reducing/Antioxidant Power (FRAP) and Gallic Acid Equivalent (GAE) assays.[6,7] The samples were diluted to equal phenolic (GAE) concentrations and analyzed another time by the FRAP assay. Solid phase extraction (SPE) was then performed on the hydrolysate of Kombucha using C-18 resin. Once the hydrolysate was added, eluents of 1% Acetic Acid, 70:30 MeOH/1% Acetic Acid, and 100% MeOH were passed through the column. These eluents were then analyzed by FRAP assays of both equal and unequal phenolic content. Reverse-phase C-18 High Performance Liquid Chromatography (HPLC) was used to separate the Kombucha, tea, and their extracts into fractions, which were then analyzed in the same manner.[8] An enzymatic assay of α-amylase was completed using the SPE eluents and Kombucha to determine the activity of amylase on the compounds in each of the samples.[9]

HPLC Method

Pump A	1% Acetic Acid	**Time Program**	**Command**	**Value**
	50%	0.01	Pump B Conc.	0
Pump B	MeOH	20.00	Pump B Conc.	10
	50%	21.00	Pump B Conc.	36
Injection Volume	300 µL	35.00	Pump B Conc.	70
SPD-10 Avp λ	280 nm	40.00	Pump B Conc.	0
		42.00	Controller Stop	

RESULTS AND DISCUSSION

Figure 1

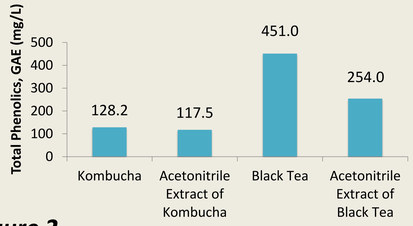


Figure 2

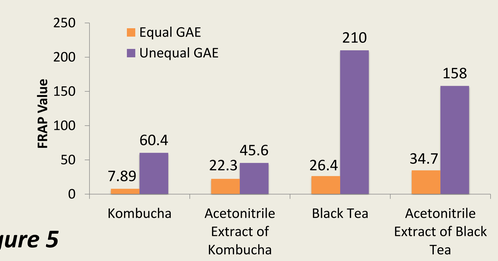


Figure 3

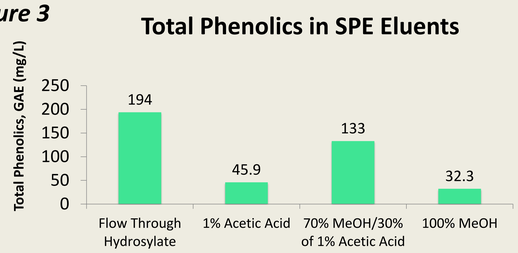


Figure 4

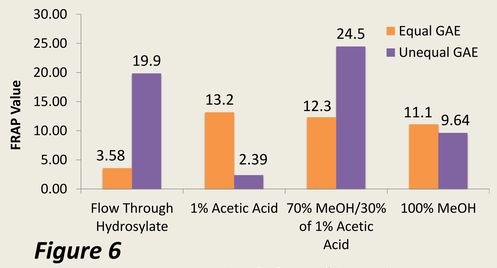


Figure 5

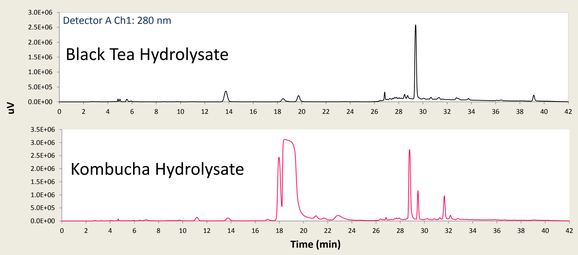
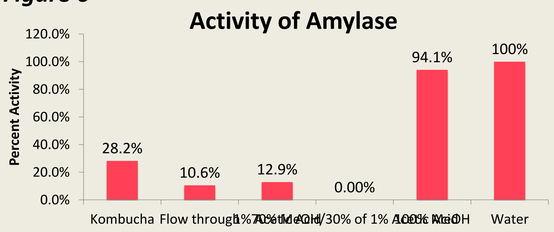


Figure 6



CONCLUSIONS

- Molecules responsible for antioxidant capacity are a mixture of both polar and non-polar hydrophobic compounds.
- Black tea has a significantly higher FRAP value at unequal GAE than Kombucha, but all samples are similar at equal phenolics. This indicates the antioxidant capacity, of the black tea especially, is heavily dependent on the phenolic content.
- Based on chromatogram from the HPLC, the molecules responsible for antioxidant activity in the tea and Kombucha appear to be different compounds. It is possible caffeic acid was present in the tea and went through a biotransformation to chlorogenic acid, an ester formed from caffeic acid and (-)-quinic acid.
- The inhibition of amylase is heavily subjective to phenolic content.

RESULTS AND DISCUSSION

It was found that the ethyl acetate extract of Kombucha contained a small antioxidant capacity, and therefore was not carried through the rest of the experiment. Kombucha, BT, and their respective acetonitrile extracts were analyzed via FRAP assays to determine antioxidant capacity. BT hade the highest FRAP value (210), followed by the acetonitrile extract of BT (158), Kombucha (60.4), and the acetonitrile of extract of Kombucha (45.6). In attempt to determine the nature of the molecule contributing to antioxidant power, the samples were analyzed for total phenolic content via a Gallic Acid Equivalent (GAE) assay. The same ranking was found for GAE as the FRAP analysis (Figure 2). The samples were then normalized to the lowest GAE concentration and reanalyzed via FRAP assay (Figure 2). It was found that the range of FRAP values was much smaller (26.81) at equal GAE than unequal (164.4). This indicated that phenolics were contributing heavily to the antioxidant capacity shown in the FRAP assay. In addition, it was also found that the antioxidant capacity of the acetonitrile extract of Kombucha was enhanced at equal GAE (22.3), being higher than Kombucha (7.89). The SPE procedure allowed for the Kombucha hydrolysate to be separated based on polarity properties. The results show that as the polarity of the mobile phase decreased (from acetic acid to MeOH), the FRAP value decreased as well (Figure 4). The range of the FRAP values at equal phenolic content; however, is only 2.1 (between the acetic acid and MeOH eluents). The extracts of Kombucha and BT were also separated using reverse phase C-18 HPLC (Figure 5) and the fractions were collected. These fractions were analyzed for Gallic Acid Equivalents; however, with such a small injection rate very few of the fractions possessed a high enough concentration of phenolics to give a reading. The activity of α-amylase when exposed to the different fractions of Kombucha was shown to be most inhibited in the 70% MeOH/30% of 1% Acetic Acid eluent, and least inhibited in the 100% MeOH fraction (Figure 6).

FUTURE PLANS

- Analyze the fractions from HPLC for antioxidant capacity.
- Compare chromatograms of samples from HPLC with chromatograms of standards such as catechin, chlorogenic acid, caffeic acid, and other phenolics.
- Determine the relationship between antioxidant capacity and amylase activity.

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