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Kelli Rush
Winona State University

Callie Gustafson
Winona State University

Katherine Seehusen
Winona State University

Ted Wilson
Winona State University

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Kelli Rush, Callie Gustafson, Katherine Seehusen, Ted Wilson and Francis Mann

Biology Department of Winona State University

Introduction

Kombucha is a type of black tea fermented by a culture of yeast and bacteria, including species such as *Sacchraromyces* and *Glucanoacetobacter*. It has risen in popularity in response to its advertised health benefits (1). Antioxidant activity is commonly advertised on food package health claims and often associated with the phenolic content of a food product. Phenolic acid content is frequently expressed as Gallic acid equivalents (GAE). Antioxidants may help protect the body from oxidative damage caused by free radicals such as H_2O_2 and O_2^-. Free radicals can oxidize lipids, proteins, DNA and low density lipoproteins (LDL)(2). Oxidation of LDL lipids results in the generation of fatty acid dienes with an absorbance at 234 nm that is central to the conjugated diene assay.

LDL consist of fatty acids, cholesterol, and a single apoB-100 protein, all of which can be oxidized, leading to increased LDL pathogenesis(3). Oxidized LDL recruit macrophages and platelets to adhere to the sides of the artery, eventually leading to an accumulation of macrophages and cholesterol. Resulting atherosclerotic plaques can slow or restrict blood flow through the artery, leading to further health complications(4). Foods high in GAE and antioxidant activity may inhibit LDL oxidation and atherosclerosis.

Kombucha tea is sold as a commercial product that includes an antioxidant claim on its food packaging label. This study sought to validate the phenolic content and antioxidant activity of GT Kombucha in acetonitrile and ethyl acetate extracts, as well as GT Kombucha titrated to pH 7.

Methods

KOMBUCHA PREPERATION: Liquid-liquid extractions were performed on Kombucha using acetonitrile and ethyl acetate to separate components. Acid hydrolysis was performed using 10% MeOH/5 M HCl to cleave glycosidic bonds.[5] Solid phase extraction was performed on the hydrolysate using C-18 resin. Eluents of 1% Acetic Acid, 70:30 MeOH/Acetic Acid, and 100% MeOH were passed through column. Reverse-phase C-18 High Performance Liquid Chromatography was used to separate fractions. Aqueous samples were prepared by using raw kombucha, spinning it down and taking the top raw product. pH adjusted samples were spun down and set to a pH of 7. Fraction phenolic concentration was estimated using a colorimetric assay and was expressed in Gallic Acid Equivalents(GAE mg/L). Quantity of phenolic concentration were expressed in each fraction.

OXIDATIVE LAG-TIME ASSAY: Blood was drawn from three volunteers in K_3 EDTA and centrifuged for plasma collection. Sequential ultracentrifugation was used to isolate LDL and three rounds of PBS dialysis were performed on the extracted LDL to remove EDTA. LDL oxidation (7 micrograms LDL protein/ml) was initiated by 10 uM copper in the presence or absence of kombucha tea extracts. Kombucha extracts were made into dilutions and standardized based off the polyphenol content assay . The dilutions compared 1:100 and 1:500 dilutions and 0.7mg/L and 0.35mg/L dilutions. The antioxidant activity measured using A_{234} at 37° C for 180 minutes.

Results

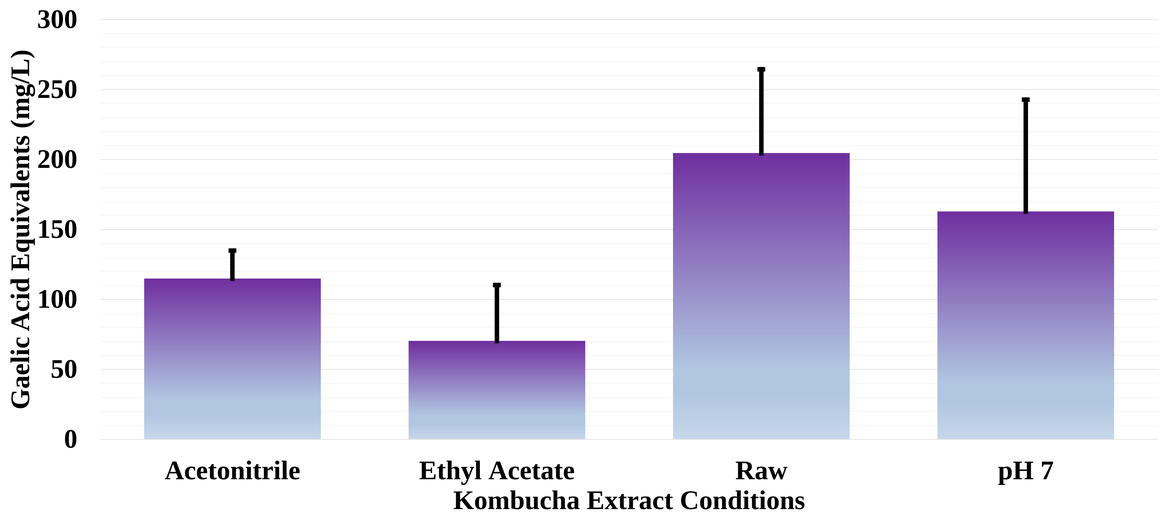

Figure 1: Phenolic content (Gallic Acid Equivalence mg/L; GAE) of Acetonitrile, ethyl acetate, aqueous and pH adjusted extracts.

Table 1: Lag-time (% control) for LDL oxidized in the presence of kombucha extracts and 10 uM Cu^{2+} Lag Times compared to Control of LDL and Cu^{2+} (no sample) of 47 ± 4.5 minutes.

Sample	1:100	1:500	1:1,000
Acetonitrile	310 ± 12%	105 ± 1%	103 ± 3%
Ethyl Acetate	135 ± 2%	102 ± 3%	104 ± 5%
pH 7	107 ± 10%	79 ± 2%	92 ± 8%

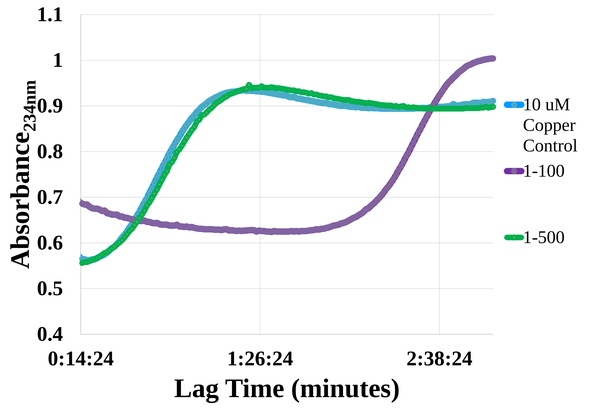

Figure 2: Lag-time for oxidation of LDL lipids (A_{234}) oxidized with of 10 uM Cu^{2+} (control) compared to GT Kombucha acetonitrile extract dilutions of 1:100 and 1:500.

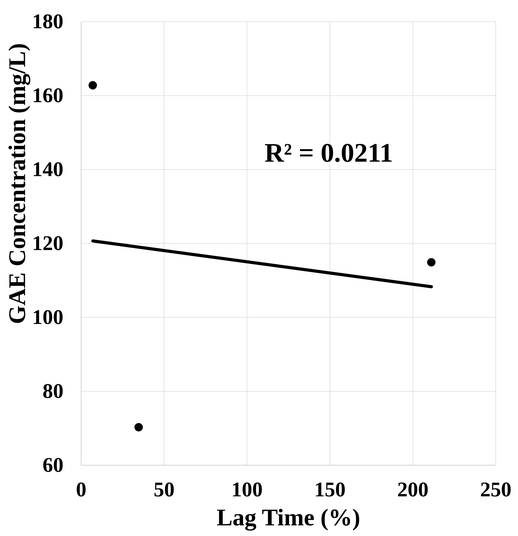

Figure 3: Correlation of GT Kombucha lag-time at 1:100 dilution and GAE for ethyl acetate, acetonitrile and pH 7.

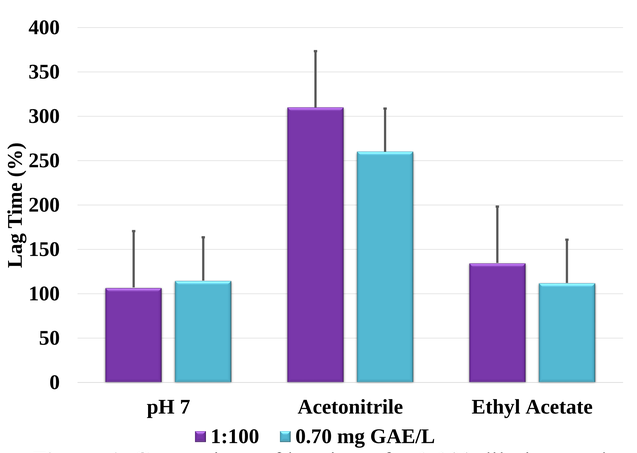

Figure 4: Comparison of lag times for 1:100 dilutions and extracts standardized to the concentration of pH adjusted (0.70 mg GAE/L). Standardizing extracts to pH adjusted concentration resulted in a slight loss of antioxidant activity, but overall did not greatly affect the lag times, especially in the case of acetonitrile. No antioxidant effects were observed when extracts were standardized to 0.35 mg GAE/L.

Main points

- Phenolic acid content (Gallic acid equivalents) of GT's Raw Kombucha was greater than acetonitrile, ethyl acetate, and pH 7 (Figure 1).
- The presence of phenolic antioxidants inhibited LDL oxidation and extended lag-times at 1:100 and 0.70 mg GAE/L, but not at 1:500 or 0.35 mg GAE/L (A_{234}).
- Measurement of antioxidant capacity of Raw Kombucha was attempted but not possible due to interference at A_{234}.
- The acetonitrile extract of GT's Raw Kombucha gave the best ability to inhibit LDL oxidation (Table 1).
- GAE concentration did not appear to be correlated with lag-times (Figure 3).
- Antioxidant activity was maintained after Kombucha extracts were standardized to 0.70 mg GAE/L (Figure 4).

Conclusions

GT Kombucha extracts inhibited LDL oxidation at a 1:100 dilution and 0.70 mg GAE/L. At 1:500 and 0.35 mg GAE/L no significant antioxidant activity was observed. Future studies may wish to isolate individual compounds from the extracts evaluated in the present study.

Acknowledgements

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References

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