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Enzymatic Effects of Pancreatic Amylase Inhibition by Kombucha Tea

Mallory Villeneuve
Winona State University

Francis Mann
Winona State University

Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project

Enzymatic effects of pancreatic amylase inhibition by Kombucha Tea

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Student Name Mallory Villeneuve

Faculty Sponsor Francis Mann

Department Chemistry

Abstract

Kombucha is a fermented tea beverage produced through use of a symbiotic culture of bacteria and yeast (SCOBY). The purported health benefits of this drink are being assessed through the use of an assay simulating breakdown of starches in the mouth. Analysis with spectrophotometry at 540 nm for porcine pancreas amylase using 0.100 mg/mL reported a Km of 0.440 mg/mL and Vmax of 0.00451 mg/min. Breakdown of starches was compared to a standard using maltose for comparison. Kombucha inhibition using IC50 inhibition analysis with a dose response curve of dilutions determined a value of 6.70x10-3 mL. Further IC50 analysis was performed using pH adjusted Kombucha and black tea samples determined values of 7.24x10-1 mL and 6.92x10-4 mL respectively. Using the Gallic Acid Equivalence method, black tea was prepared with similar phenolic concentration to that used in Kombucha during the fermentation process. In adjusting the pH of Kombucha and black tea samples, an acidified solution demonstrated the highest inhibitory function on pancreatic amylase. This data provides relevant information related to the absorption of starches in the mouth during intake of the beverage. The slowed breakdown of carbohydrates to monosaccharides could be pertinent in the overall health and digestive effects of Kombucha during consumption.

The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature Mallory Villeneuve Date 5/1/14

Faculty Sponsor Signature Francis Mann Date 05/01/14

Enzymatic effects of pancreatic amylase inhibition by Kombucha tea

Mallory Villeneuve
Department of Chemistry
Winona State University
Advisor: Francis Mann
Spring 2014

Abstract:

Kombucha is a fermented tea beverage produced through use of a symbiotic culture of bacteria and yeast (SCOBY). The purported health benefits of this drink are being assessed through the use of an assay simulating breakdown of starches in the mouth.[1] Analysis with spectrophotometry at 540 nm for porcine pancreas amylase using 0.100 mg/mL reported a K_m of 0.440 mg/mL and V_{max} of 0.00451 mg/min. Breakdown of starches was compared to a standard using maltose for comparison.[2] Kombucha inhibition using IC_{50} inhibition analysis with a dose response curve of dilutions determined a value of $6.70x10^{-3}$ mL. Further IC_{50} analysis was performed using pH adjusted Kombucha and black tea samples determined values of $7.24x10^{-1}$ mL and $6.92x10^{-4}$ mL respectively. Using the Gallic Acid Equivalence method, black tea was prepared with similar phenolic concentration to that used in Kombucha during the fermentation process. In adjusting the pH of Kombucha and black tea samples, an acidified solution demonstrated the highest inhibitory function on pancreatic amylase. This data provides relevant information related to the absorption of starches in the mouth during intake of the beverage. The slowed breakdown of carbohydrates to monosaccharides could be pertinent in the overall health and digestive effects of Kombucha during consumption.[3]

Introduction:

Kombucha has been utilized as a fermented tea beverage for hundreds of years, originally in China before spreading to the rest of the world. With this beverage came copious amounts of proposed health claims and medicinal benefits.[1,5] Kombucha contains a tea source generally composed of black or green tea in a sucrose solution that is fermented with a symbiotic culture of bacteria and yeast (SCOBY). This effectively creates a bubbly, fermented drink with high antioxidant levels in an acidic environment (pH ~2.68 for the commercial Kombucha studied) containing live cultures of bacteria.

During the first step of human digestion, starches are broken down in the mouth by two essential enzymes, human salivary α-amylase and α-glucosidase. This study focused on the degradation of dietary starches into glucose and maltose by α-amylase. This process influences the absorption of carbohydrates and inhibition of amylase can reduce the effects of hyperglycemia post food intake.

These inhibitory effects are commonly seen in phenolics and certain antioxidant structures have been linked to varying levels of inhibition in amylase.[3] Fruits containing large concentrations of phenolic containing compounds such as tannins in strawberry and raspberry extracts are known to promote α-amylase inhibition, thereby affecting the starch digestion ocurring.[4] Acids are also known inhibitors of enzymes through their ability to manipulate side chain and amino acid structures of intramolecular forces within a compound, affecting the substrate and binding site interaction.

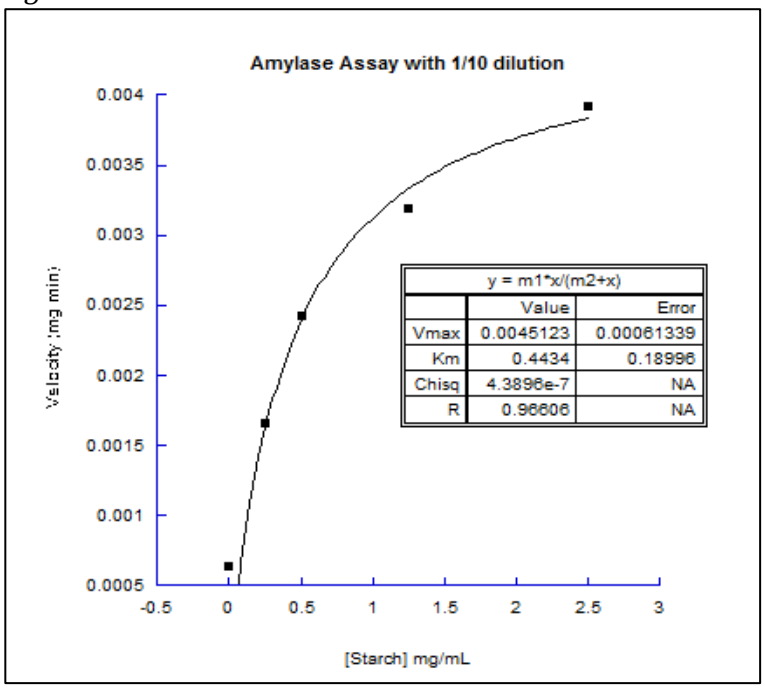
Experimental Methods:

Initial laboratory procedure utilized an Enzymatic Assay of α-amylase.[2] Porcine pancreas amylase was substituted as a suitable reagent for the assay with a starch substrate. The enzyme was prepared initially to 1.0 unit/mL. A standard of maltose was used to correlate freed sugars to the breakdown of starch by pancreatic amylase. Optimization of the assay involved a 10-fold dilution of the pancreatic amylase in order to fully saturate the enzyme. Inclusion of varying inhibition sources were added directly to the substrate samples before the enzymatic reaction was initiated. IC_{50} curves were constructed in replicate with controls and background data performed in parallel to each data set. A dose-response curve using the program Kaleidagraph was constructed for analysis of IC_{50} data. Incubation time was increased to 5 minutes. Due to the glycosides contained in the KT itself, background data using amylase with no starch was used for adjustments and allowed for analysis of enzyme activity due solely to the interaction of the starch substrate. Colorimetric analysis was performed using Spectrophotometry at 540 nm. G.T.'s original Kombucha was used for all Kombucha dilutions and tea solutions were constructed using a Gallic Acid Equivalence (GAE) method.[6] Brewed black tea was diluted to contain the same concentration of phenolics as the original KT solution. pH adjusted solutions of tea and Kombucha used negligible volumes of NaOH and Acetic Acid for adjustments.

Results:

After optimizing the pancreatic amylase assay, the following data was used for determination of the enzyme binding constant.

Figure A.



The following IC_{50} curves were then plotted using a variety of inhibitors with adjusted pH values as described on Table 1.

Figure B.

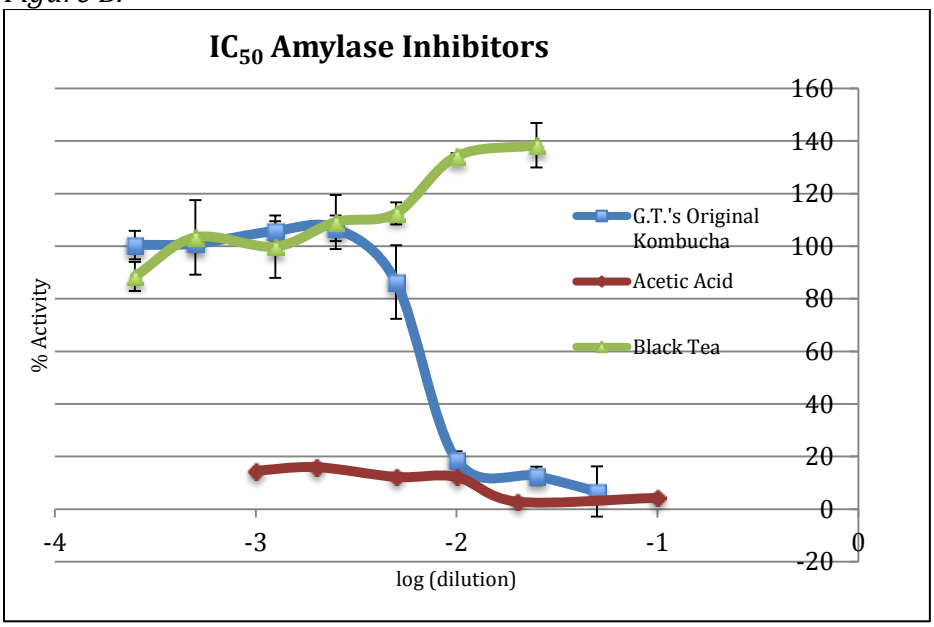


Figure C.

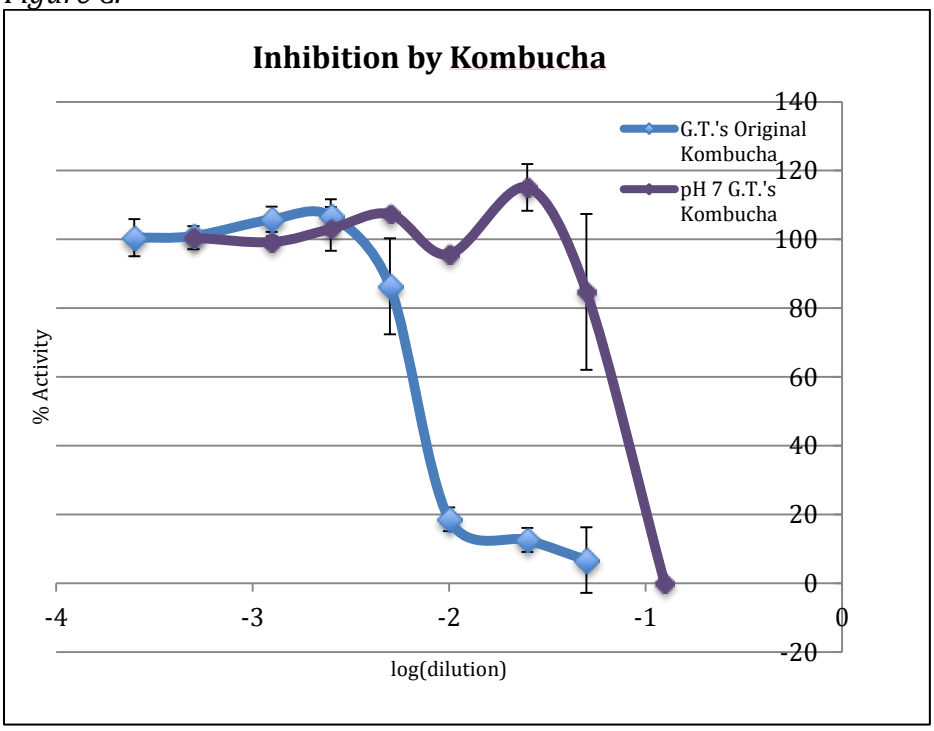
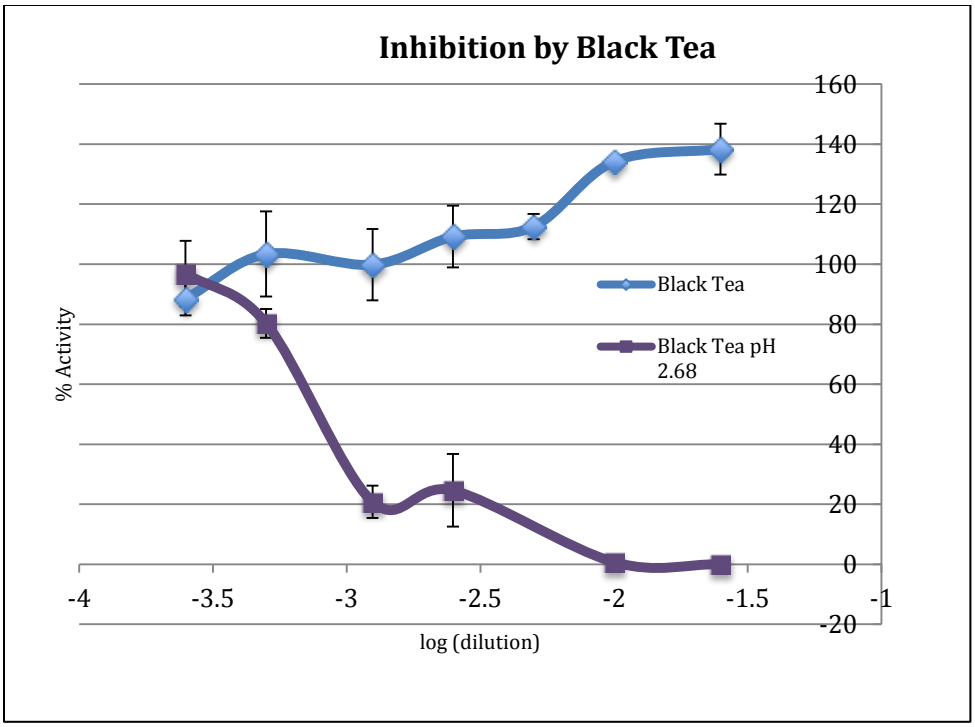


Figure D.



A table of values was constructed demonstrating the pH value correlated to IC_{50} concentration determined by dose-curve analysis.

Table 1.

Inhibitor	pH	IC_{50}	R^2
G.T.'s Original Kombucha	2.68	6.70×10^{-3} mL/U amylase	0.99
G.T.'s Kombucha	7.00	7.24×10^{-1} mL/U amylase	0.96
GAE Black Tea	5.06	significant activity	-
Black Tea	2.68	6.92×10^{-4} mL/U amylase	0.96
Acetic Acid	2.40	$<<2.50 \times 10^{-4}$ mL/U amylase	-

Discussion:

Using starch concentrations ranging from 0 to 5 mg/mL, a K_m value of 0.441 mg/mL was determined. This K_m value was used to setup an IC_{50} system testing the inhibition capacity of Kombucha and black tea. Figure C shows the IC_{50} curves for the original KT solution and the pH adjusted solution, exhibiting less inhibition in comparison. The original black tea shown in Figure D. showed no inhibition, but increased inhibition when the pH was lowered with acetic acid. This value demonstrates more inhibition than the original Kombucha beverage likely due to the presence of acetic acid to achieve a similar pH. Figure B. relates the inhibition due to acetic acid, a possible biproduct of the fermentation process, to the Kombucha and tea solutions. The low activity levels of acetic acid with amylase

could demonstrate some contribution of the inhibition found in the original Kombucha, however more acids must be produced in biotransformation of the molecules present during the fermentation process to exhibit the trend seen in Figure B.

Conclusion:

Kombucha was shown as a significant inhibitor of pancreatic amylase, largely due to acidic components present. Inhibition by Kombucha contrasted with both the black tea and acetic acid inhibition trends indicating transformation of the molecular composition during fermentation. Significant inhibition in varying solutions was demonstrated in highly acidified pH levels overall. Similar to other amylase inhibitors, use of Kombucha as a product limiting carbohydrate absorption may contribute to proposed health benefits and regulation of hyperglycemia. Kombucha is desirable in the health market for this benefit in addition to probiotic and antioxidant properties.

References:

(1) Sreeramulu, G.; Zhu, Y.; Knol, W. Kombucha Fermentation and Its Antimicrobial Activity. *J. Agric. Food Chem.* **2000**, *48*, 2589–2594.
(2) Enzymatic Assay of α-AMYLASE (EC 3.2.1.1) http://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-a-amylase.html (accessed Feb 22, 2014).
(3) Lo Piparo, E.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M.; Chou, C. J. Flavonoids for Controlling Starch Digestion: Structural Requirements for Inhibiting Human α-Amylase. *J. Med. Chem.* **2008**, *51*, 3555–3561.
(4) McDougall, G. J.; Shpiro, F.; Dobson, P.; Smith, P.; Blake, A.; Stewart, D. Different Polyphenolic Components of Soft Fruits Inhibit α-Amylase and α-Glucosidase. *J. Agric. Food Chem.* **2005**, *53*, 2760–2766.
(5) Vīna, I.; Semjonovs, P.; Linde, R.; Deniņa, I. Current Evidence on Physiological Activity and Expected Health Effects of Kombucha Fermented Beverage. *J. Med. Food* **2014**, *17*, 179–188.
(6) Kim, D.-O.; Jeong, S. W.; Lee, C. Y. Antioxidant Capacity of Phenolic Phytochemicals from Various Cultivars of Plums. *Food Chem.* **2003**, *81*, 321–326.