

Introduction

- Kombucha is a fermented tea, where a yeast fermentation of sugar to alcohol is followed by a bacterial fermentation of alcohol to acetic acid.
- Kombucha fermentation is the conversion of sucrose to glucose, followed by the metabolization of ethanol and carbon dioxide. Ethanol further metabolizes into acetaldehyde, and forms acetic acid.
- The combination of the alcohol content and acetic acid will present a unique challenge in the creation of an accurate nutrition facts label, as the conversion rate of sucrose to ethanol has never been studied, and acetic acid affects traditional methods accuracy such as % brix.
- Carbohydrates are calculated at 4 calories/gram, while alcohol is calculated at 7 calories/gram.

Objective

- The objective of this study was to exhibit the need for development of new methods of carbohydrate analysis, and demonstration of the inaccuracy of Kombucha testing in the Food Industry compared to the producer's label.

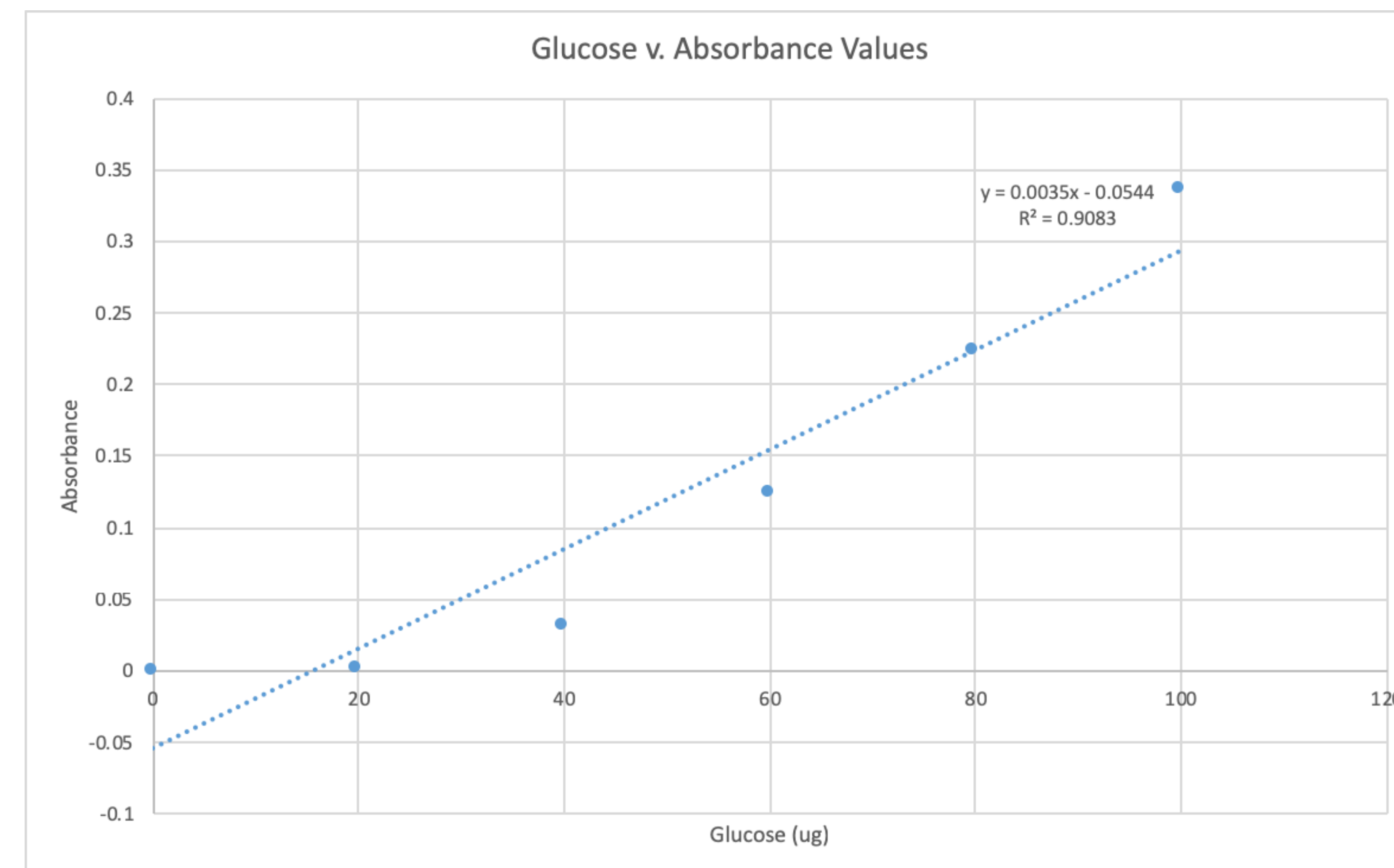
Materials & Methods

- Phenol-Sulfuric Acid Method for Total Carbohydrates
 - Prepare a standard curve from known concentrations of glucose stock solution (100 mg glucose/L water), table listed below.

	µg Glucose/2 ml					
	0	20	40	60	80	100
ml glucose stock solution	0	0.2	0.4	0.6	0.8	1.0
ml dd water	2.0	1.8	1.6	1.4	1.2	1.0

- Dilute Kombucha by 1:2000, by pipetting 1 mL of beverage into 1000-mL flask, and then combining 1 mL diluted solution and 1 mL distilled water in test tube.
- Add .05 mL of 80% phenol solution to each tube, vortex.
- To each tube, add 5 mL of concentrated Sulfuric Acid. Vortex and allow reaction to occur for 10 minutes, then waterbath at 25 C for 10 minutes.
- Pour sample into cuvette, and read absorbance in spectrophotometer at 490 nm. Construct standard curve using Excel, and calculate glucose value from best-fit equation.
- Measurement of % Brix
 - Using a refractometer, inoculate the prism glass with sample of Kombucha.
 - Using reading on scale, multiply by the serving size to get value of soluble solids in Kombucha.
- Density Measurement
 - Using a graduated cylinder, tare scale and fill with 100 mL of Kombucha.
 - Calculate Density by taking mass/volume, and compare with known values from % Brix to Specific Gravity Table (Table 2) to get value of % soluble solids in Kombucha

Results



Graph 1: Glucose vs Absorbance Values

Table 2: Specific Gravity to % Brix (Processing Procedures for Canned Food Products)

Degrees Brix (% by Weight of Sugar)	Specific Gravity (20 C)	Lbs of Sugar in 1 U.S. Gallon of Solution at 20 C)
0	1	0
1	1.004	0.08
2	1.008	0.17
3	1.012	0.25
4	1.016	0.34
5	1.02	0.42
6	1.024	0.51
7	1.028	0.6
8	1.032	0.69
9	1.036	0.78
10	1.04	0.87

Table 3: Statistical Analysis of Carbohydrate Testing

	Phenol Sulfuric Acid Method v. % Soluble Solids	Phenol Sulfuric Acid Method v. Density	% Soluble Solids v. Density
Standard Deviation	5.444722215	5.444722215	0
Average	18.25	18.25	14.4
Coefficient of Variation (%)	29.83409433	29.83409433	0
Variance	29.645	29.645	0

Table 1: Comparison of Carbohydrate Testing Methods

Column1	Column2	Column3
Testing Method	Sugars (g)	Calculated Calories (240 mL)
Phenol Sulfuric Acid Method (Glucose)	22.1	90 calories
% Soluble Solids	14.4	60 calories
Density (Sucrose)	14.4	60 calories

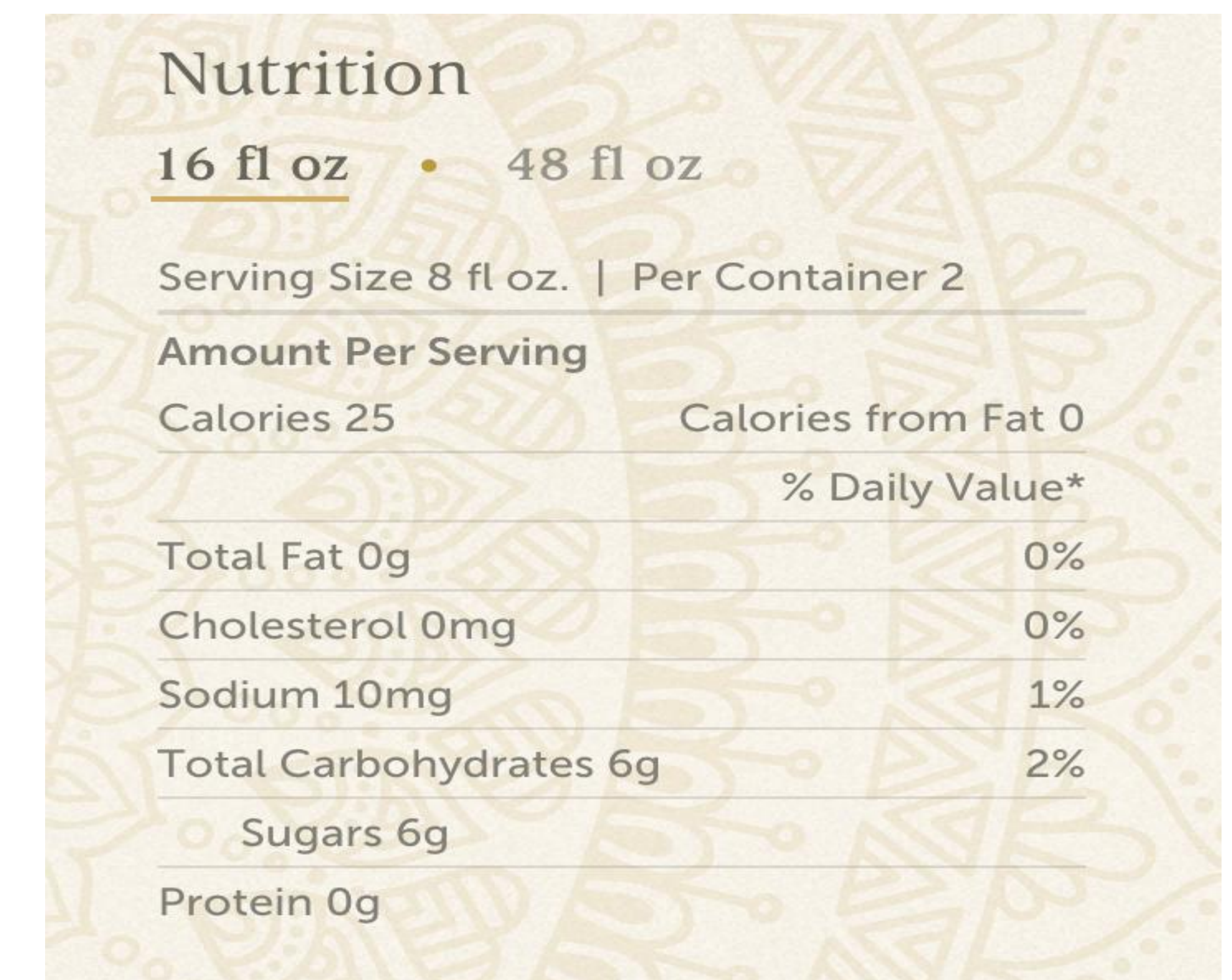


Figure 1: GT Original Kombucha Nutrition Facts Panel

Conclusions

- The Density and % Soluble Solids methods provide estimation of the sugar content at 14.4 grams. The alcohol content and acetic acid do not appear to be present in high enough amounts to significantly effect testing results.
- Phenol Sulfuric Acid Method is very tedious, and relies on accuracy of reaction rates. Very hard to replicate results and ensure accuracy, relies on standard curve.
- Further study should be conducted into the study of the conversion of glucose to ethanol, as Kombucha must be below 0.5% in order to not be regulated by the Alcohol and Tobacco Tax and Trade Bureau. Gas Chromatography or Anton Paar Beer Alcoyzer are highly accurate methods that should be explored.

References

- Featherstone S (2016) A Complete Course in Canning and Related Processes, 14th edn. Elsevier, Boston, MA
- Kumar, V., & Joshi, V. K. (2016). Kombucha: Technology, Microbiology, Production, Composition and Therapeutic Value. *International Journal of Food and Fermentation Technology*, 6(1), 13-24. Retrieved August 26, 2018.
- Nielsen SS (2003) Phenol-Sulfuric Acid Method for Total Carbohydrates. Food Analysis Laboratory Manual Food Science Text Series 39–44. doi: 10.1007/978-1-4757-5250-2_6
- Nummer, B. A., Dr. (2013). Kombucha Brewing Under the Food and Drug Administration Model Food Code: Risk Analysis and Processing Guidance. *Journal of Environmental Health*, 76(4), 8-11. Retrieved August 23, 2018.