# Determination of Carbohydrates in Kombucha using HPAE-PAD

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## ABSTRACT

**Purpose:** In recent years kombucha has become popular in the US due to its reported health benefits. For quality control, carbohydrate measurement in kombucha might be necessary.

**Methods:** The method uses the Thermo Scientific™ Dionex™ CarboPac™ PA20 column with electrolytically generated hydroxide eluent and a 15 mil gasket for the gold working electrode to separate and detect simple carbohydrates in kombucha.

**Results:** This study developed an accurate method for determining simple carbohydrates in kombucha using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD).

## INTRODUCTION

Kombucha is any of a variety of fermented, lightly effervescent sweetened black or green tea drinks that are commonly intended as functional beverages for their reported health benefits. Kombucha is uniquely fermented simultaneously with yeasts and bacteria in an aerobic environment. In recent years kombucha has become popular in the U.S. due to its reported health benefits. It is said to help regulate blood sugar and possibly help with high blood pressure and high cholesterol, making it a drink of interest to diabetics.

Sucrose is the most common carbon source in kombucha fermentation. During fermentation, it is believed that most of the sugar is consumed by the bacteria and yeast, so it has minimal effect on blood sugar levels in the body. Generally, sucrose is biochemically converted into fructose and glucose, which are metabolized to gluconic acid and acetic acid. These metabolites are present in the drink.[1] Though most of the sugar is converted into other components during the fermentation process, some still remains in the finished tea.

## MATERIALS AND METHODS

**Sample Preparation**

Centrifuge kombucha samples at 6500–7500 g for 15 min. Pass the supernatant through a Nalgene syringe filter (0.2 µm) and dilute 1:500 with DI water prior to analysis.

**Test Method(s)**

| Conditions | |
|---|---|
| Columns: | Thermo Scientific Dionex CarboPac PA20 analytical column (3×150 mm) |
| Eluent Source: | Thermo Scientific Dionex EGC III KOH with CR-ATC 500 |
| Eluent: | Potassium hydroxide |
| Isocratic: | 100 mM KOH from -15 min to -10.05 min only for column wash, 10 mM KOH from -10.00 min to 0 min for equilibrium, 10 mM KOH from 0 min to 15 min |
| Flow Rate: | 0.5 mL/min |
| Injection Volume: | 10 µL |
| Temperature: | 30 °C (column and detector compartments) |
| Backpressure: | ~2700 psi |
| Detection: | Pulsed amperometric |
| Background: | ~40 nC |
| Working Electrode: | Disposable Au on PTFE electrode |
| Electrochemical Cell Gasket: | 15 mil* |
| Reference Electrode: | pH, Ag/AgCl, Ag mode |
| Noise: | 30 - 60 pC |

*** 1 mil = 0.001 inches**

**Data Analysis**

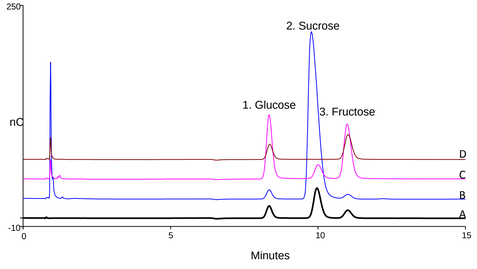
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software version 7.2.

## RESULTS

**Separation**

Figure 1, Chromatogram A shows the separation of three carbohydrates on a Dionex CarboPac PA20 column. Using a hydroxide isocratic elution, glucose, sucrose, and fructose are fully resolved. The total separation time is 15 min with an additional 5 min wash and 10 min equilibration prior to sample injection. The column wash ensures stable retention times during analysis of kombucha samples. Three kombucha samples were purchased from the store and designated A ,B, and C.

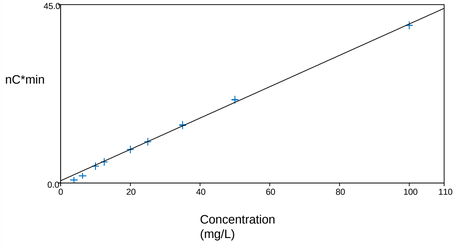
**Figure 1. Separation of glucose, sucrose, and fructose on a Dionex CarboPac PA20 column in (A) a mix of standards, glucose (4.5 mg/L), sucrose (25 mg/L), and fructose (5 mg/L), (B) Kombucha A with 100-fold dilution, (C) Kombucha B with 500-fold dilution and (D) Kombucha C with 500-fold dilution.**




**Linear Range**

Linearity for three sugars was determined by injecting calibration standards in triplicate, glucose ranging from 0.2–50 mg/L, sucrose from 10–100 mg/L, and fructose from 0.2–100 mg/L. Glucose, sucrose, and fructose were linear with the coefficients of determination at 0.9997, 0.9992, and 1.000, respectively (Table 2).

**Figure 2. Calibration plot for sucrose in the concentration range of 2.5–100 mg/L. A linear curve in the narrower range (10–100 mg/L) was used for sample analysis.**




**Sample analysis**

All three target analytes are present in Kombucha A and B, on the other hand, only glucose and fructose are found in Kombucha C. When using a 2 mil working electrode gasket, different dilutions are needed for the three samples to have all sample sugars fall within their linear calibration range. Therefore, using the 15 mil gasket simplifies the method and reduces the amount of dilution needed for some samples. The amount of sugars in the samples measured in summarized in Table 1.

**Table 1. Carbohydrate quantification in kombucha.**

| Sample | Glucose | Sucrose | Fructose |
|---|---|---|---|
| | g/L (amount present after correcting for the dilution factor) | | |
| **Kombucha A** | 0.266 | 22.0 | 0.319 |
| **Kombucha B** | 12.1 | 5.95 | 17.8 |
| **Kombucha C** | 2.77 | N.A. | 7.75 |

**Precision**

For our method, the peak area and retention time precisions were determined for six replicate injections of a mixture of sugar standards (Table 2). The high precisions suggest that our method can be used for the analysis of kombucha for these three carbohydrates.

**Table 2. Method precisions and calibration.**

| Analyte | RT (min) | RT RSD (%) | Area (nC*min) | Peak Area RSD (%) | Coefficient of Determination ($r^2$) |
|---|---|---|---|---|---|
| **Glucose** | 8.36 | 0.04 | 3.386 | 0.25 | 0.9997 |
| **Sucrose** | 9.99 | 0.00 | 10.459 | 0.27 | 0.9992 |
| **Fructose** | 11.03 | 0.04 | 2.791 | 0.73 | 1.000 |

**Accuracy**

The accuracy of our method on the Dionex CarboPac PA20 column was verified by determining recoveries of sugars in spiked kombucha samples (Table 3). The average recovery for three sugars ranged from 80 to 126%, indicating that this method can accurately determine carbohydrates in kombucha samples.

**Table 3. Recovery of glucose, sucrose, and fructose.**

| | Kombucha A (diluted 500 fold) | | | Kombucha B (diluted 500 fold) | | | Kombucha C (diluted 500 fold) | |
|---|---|---|---|---|---|---|---|---|
| **Recovery (%)** | Glucose | Sucrose | Fructose | Glucose | Sucrose | Fructose | Glucose | Fructose |
| **20% addition** | 98 | 82 | 83 | 97 | 126 | 105 | 102 | 94 |
| **50% addition** | 93 | 90 | 112 | 94 | 116 | 98 | 100 | 97 |
| **100% addition** | 104 | 90 | 107 | 90 | 118 | 94 | 97 | 97 |
| **150% addition** | 80 | 96 | 107 | 99 | 111 | 98 | 101 | 93 |

**Comparison of the 15 mil Gasket to the 2 mil Gasket**

The thicker gasket extends the linear calibration range to higher concentrations. Increasing the gasket thickness decreases the linear flow rate at the electrode. Table 4 summarizes the decrease in response of glucose, sucrose, and fructose with the 15 mil gasket relative to the 2 mil gasket. Due to the increased linear range of the method, carbohydrates present in major and minor amounts can generally be measured using a 500-fold dilution instead of a greater dilution (or multiple dilutions) needed with the 2 mil gasket.

**Table 4. % Decrease in response with the 15 mil gasket relative to the 2 mil gasket.**

| Glucose (mg/L) | | Sucrose (mg/L) | | Fructose (mg/L) | |
|---|---|---|---|---|---|
| 0.2 | 30% | 10 | 30% | 0.2 | 49% |
| 0.5 | 31% | | | 0.5 | 55% |
| 1.5 | 35% | 20 | 45% | 1.5 | 60% |
| 4.5 | 38% | | | 5 | 62% |
| 12 | 39% | 50 | 64% | 16 | 65% |
| 25 | 47% | | | 50 | 74% |

## CONCLUSIONS

- We developed a method for the accurate determination of glucose, sucrose, and fructose in kombucha samples.
- The method uses the Dionex CarboPac PA20 column with an electrolytically generated hydroxide eluent and a thicker gasket for the working electrode.
- The method is shown to have a linear range suitable for handling the wide range of sugar concentrations among the three kombucha brands analyzed with minimal sample treatment.

Note: See Thermo Scientific Application Note 1152 for more details on method setup.[2]

## REFERENCES

1. Sreeramulu G.; Zhu Y.; Knol W. Kombucha fermentation and its antimicrobial activity. *J. Agric. Food Chem.* **2000**, 48 (6): 2589–2594.
2. Thermo Scientific Application Note 1152: Determination of Carbohydrates in Kombucha Using HPAE-PAD. Sunnyvale, CA. **2016**.

## TRADEMARKS/LICENSING



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