Determination of Organic Acids in Kombucha by Ion Chromatography

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ABSTRACT

Purpose: To monitor the organic acid profile of kombucha. The determination of organic acids plays an important role in revealing the beneficial effects of kombucha. Organic acids also affect the flavor and taste of the drink.

Methods: This work took advantage of the 4 µm particle-size Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm column combined with a high-pressure IC system for optimal separation and quantification of organic acids and inorganic anions in kombucha samples.

Results: The results showed that acetic acid is the predominant organic acid, and gluconic acid is another major organic acid produced as a result of the kombucha fermentation process. It is often claimed that kombucha contains glucuronic acid, a potent detoxifying compound that the liver produces. In our study, no glucuronic acid was detected in kombucha.

INTRODUCTION

Fermentation of sugared tea with a symbiotic culture of acetic acid bacteria and yeast (tea fungus) yields kombucha tea, which is consumed worldwide for its refreshing and reported beneficial properties to human health. The beneficial effects of kombucha are attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics, and a variety of micronutrients produced during fermentation.[1]

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 1:20 with DI water prior to analysis.

Test Method(s)

Conditions			
Columns:	Dionex IonPac AS11-HC-4 µm Guard, 2 × 50 mm (P/N 078036) Dionex IonPac AS11-HC-4 µm Analytical, 2 × 250 mm (P/N 078035)		
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with CR-ATC 500		
Eluent A:	DI Water		
Eluent B:	Methanol (CH_3OH)		
Gradient:		Pump:	Multi-Step Gradient
Time (min)	KOH (mM)	Time (min)	B (%)
-2.000	1	-2.000	0
0.000	1	0.000	0
10.070	1	10.700	8
10.071	1	20.000	8
24.000	15	25.000	15
24.010	15	31.000	15
35.000	27	33.000	8
40.000	60	33.010	0
44.000	60	45.000	0
44.010	1		
45.000	1		
Flow Rate:	0.45 mL/min		
Injection Volume:	2.5 µL		
Temperature:	45 °C (column compartment), 35 °C (detector compartment)		
System Backpressure:	~4100 psi (1 mM KOH/0% CH3OH), ~4800 psi (20 mM KOH/15% CH3OH)		
Detection:	Suppressed Conductivity, Dionex AERS 500 Electrolytically Regenerated Suppressor (2 mm), AutoSuppression, external water mode, external water flow rate (equal to the eluent flow rate)		
Background Conductance:	~ 0.5 µS		
Run Time:	47 min		

Data Analysis

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

RESULTS

Separation

Twenty anions were separated on the Dionex IonPac AS11-HC-4µm column set (Figure 1). The method starts with a low eluent concentration (1 mM KOH) to separate the weakly retained anions, such as quinate, fluoride, gluconate, lactate, and acetate. Using a Dionex IonPac AS11-HC-4µm column set, gluconate and fluoride co-eluted. The addition of methanol did not improve the separation. Gluconate and fluoride were separated on a Dionex IonPac AS19-4µm column set. (Figure 2) This analysis revealed that fluoride is either absent or present at very low concentrations in kombucha samples, and, therefore gluoconate and fluoride separation is not required for this analysis.

Figure 1. Separation of 20 organic and inorganic anions on the Dionex IonPac AS11-HC-4 µm column in (A) Kombucha A with 20-fold dilution, (B) Kombucha B with 20-fold dilution, (C) Kombucha C with 20-fold dilution, and (D) a mix of 20 anion standards (complete conditions shown in the Conditions section).

Peaks	Standard (mg/L)	A (mg/L)	B (mg/L)	C (mg/L)		Standard (mg/L)	A (mg/L)	B (mg/L)	C (mg/L)
1. Quinate	5	2.18	0.684	1.45	11. Bromide	5	—	—	—
2. Fluoride	0.5	—	—	—	12. Nitrate	5	—	—	—
3. Gluconate	5	1.46	9.20	111	13. Succinate	5	1.03	12.8	2.09
4. Lactate	5	2.31	5.58	1.15	14. Malate	5	1.57	7.41	4.27
5. Acetate	5	136	39.0	168	15. Malonate	5	n.a.	n.a.	n.a.
6. Propionate	5	35.4	—	—	16. Tartrate	5	n.a.	n.a.	n.a.
7. Pyruvate	5	—	7.97	—	17. Sulfate	2.5	1.43	1.08	0.300
8. Chloride	1.25	0.841	0.994	0.505	18. Oxalate	5	0.319	0.313	1.06
9. Glucuronate	5	—	—	—	19. Phosphate	7.5	0.298	—	0.476
10. Nitrite	2.5	—	—	—	20. Citrate	10	16.0	3.85	1.65

'–"- < LOQ or not detected
n.a. - not applicable

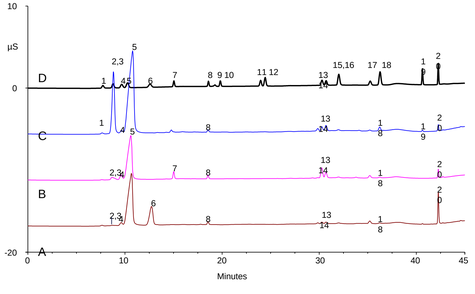
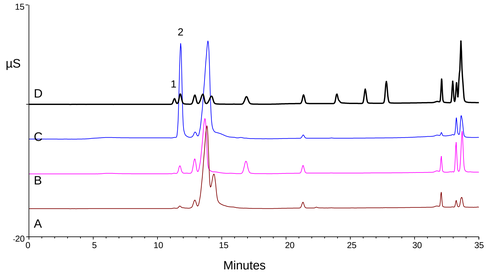


Figure 2. Separation of gluconate and fluoride on the Dionex IonPac AS19-4µm column.

Column:	Dionex AS19-4µm, Analytical, 4 x 250 mm Dionex AG19-4µm, Guard, 4 x 50 mm
Eluent Source:	Dionex EGC 500 KOH cartridge with CR-ATC 500
Gradient:	5 mM KOH (-5-15 min), 5-30 mM (15-25 min), 30-60 mM (25-33 min), 60 mM (33-35 min)
Column Temperature:	50˚C
Flow Rate:	0.8 mL/min
Inj. Volume:	10 µL
Detection:	Suppressed conductivity, Dionex AERS 500 suppressor, 4 mm, AutoSuppression, recycle mode
Samples:	A: Kombucha A with 20-fold dilution; B: Kombucha B with 20-fold dilution; C: Kombucha C with 20-fold dilution; D: a mix of standards, fluoride (0.5 mg/L) and gluconate (5 mg/L)
Peak:	1. Fluoride, 2. Gluconate



Calibration, Limit of Detection, and Limit of Quantitation

Gluconic, lactic, acetic, pyruvic, succinic, malic, oxalic, and citric acids were determined by injecting calibration standards in triplicate.

A signal-to-noise ratio 3:1 is generally considered acceptable for estimating the limit of detection (LOD), and a signal-to-noise ratio 10:1 for limit of quantification (LOQ). The LOD and LOQ were then calculated from the average peak height of five injections of 0.2 mg/L each of the standards. The results of the calibration, LOD, and LOQ are summarized in Table 1.

Table 1. Method calibration, LOD, and LOQ.

Compound	Range (mg/L)	Coefficient of Determination (r^2)[a]	LOD[b] (mg/L)	LOQ[c] (mg/L)
Gluconate	0.2–150	0.9996	0.042	0.14
Acetate	5–400	0.9997	0.006	0.02
Lactate	1–100	0.9998	0.012	0.04
Pyruvate	1–100	0.9998	0.021	0.07
Succinate	1–100	0.9999	0.057	0.19
Malate	1–100	0.9996	0.020	0.07
Oxalate	0.2–20	1.000	0.027	0.09
Citrate	1–100	0.9996	0.013	0.04

[a]Quadratic fit
[b]LOD=3×S/N
[c]LOQ=10×S/N

Sample analysis

In Figure 1, Chromatograms A, B, and C show the analysis of three brands of kombucha. Although all the samples are all sold as kombucha tea, there are differences in their organic acid composition. The various organic acids were identified by comparing their retention times with those of standards. Acetic acid bacteria from kombucha produce acetic acid as one of the main metabolites, when sucrose is used as a carbon source. It is the predominant organic acid found in kombucha. Gluconic acid is also one of the major organic acids produced as a result of the kombucha fermentation process on the traditional substrate, a black or green tea extract sweetened with 5–8% sucrose. Acetic acid produces an astringent and acidic flavor, while gluconic acid has a mild flavor.

Lactic acid, succinic acid, malic acid, oxalic acid, and citric acid are not characteristic compounds for kombucha tea, but they are found in the three kombucha samples. Pyruvic acid is present in Kombucha B, but not found in Kombuchas A and C. Propanoic acid is found only in Kombucha A.

It is often claimed that kombucha contains glucuronic acid, a potent detoxifying compound that the liver produces. In reality, no credible laboratory analysis of kombucha tea has found glucuronic acid. In our study, no glucuronic acid was detected in kombucha.

Precision

The precision of an analytical procedure is usually expressed as the relative standard deviation (RSD) of a series of measurements. The retention time RSDs and the peak area RSDs of the three representative analytes are within 0.1% and 7% respectively (Table 2), indicating good method precision at these analyte concentrations.

Table 2. Method Precisions.

Analyte	RT (min)	RT RSD	Area (nC*min)	Peak Area RSD
Acetate	10.14	0.10	5.46	1.87
Succinate	30.27	0.01	0.03	7.19
Citrate	42.27	0.01	0.03	1.13

Accuracy

The accuracy of our method on the Dionex IonPac AS11-HC-4µm column was verified by determining recoveries of acetate, succinate, and citrate in spiked kombucha samples (Table 3). The average recovery for three organic acids ranged from 81 to 115%, indicating that this method can accurately determine organic acids in kombucha samples.

Table 3. Recovery of acetate, succinate, and citrate.

	Kombucha A (diluted 20 fold)			Kombucha B (diluted 20 fold)			Kombucha C (diluted 20 fold)		
Recovery (%)	Acetate	Succinate	Citrate	Acetate	Succinate	Citrate	Acetate	Succinate	Citrate
20% addition	87	109	101	115	95	107	81	92	92
50% addition	99	98	103	109	97	109	94	101	107
100% addition	101	97	100	104	92	108	99	97	110
150% addition	n.a	103	97	n.a	105	106	n.a	96	109

CONCLUSIONS

- This study presents profiles of the inorganic anions and organic acids together with an accurate determination of organic acids in kombucha samples.
- The method uses a Dionex IonPac AS11-HC-4µm column, which is ideal for separating a wide range of organic acids and inorganic anions present in complex samples, such as kombucha tea, in combination with a HPIC system.
- The suppressed conductivity detection offers high sensitivity for the anions, including various organic acids. The specificity and sensitivity of this method allow simple sample treatments without complex procedures such as extraction and/or derivatization.

Note: See Thermo Scientific Application Note 1157 for more details on method setup.[2]

REFERENCES

1. Vijayaraghavan R, Singh M, Rao PVL, Bhattacharya R, Kumar P, Sugendran K, Kumar O, Pant SC, Singh R . Subacute (90 days) oral toxicity studies of Kombucha Tea. *Biomedical and Environmental Sciences* **2000**, 13: 293–299.
2. Thermo Scientific Application Note 1157: Determination of Organic Acids in Kombucha Using a High-Pressure Ion Chromatography System. Sunnyvale, CA. **2016**.

TRADEMARKS/LICENSING

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