Food Chemistry 111 (2008) 784–788

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of anionic minerals in black and kombucha tea using ion chromatography

Sangita D. Kumar [a,*], G. Narayan [b], S. Hassarajani [c]

[a] Analytical Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India
[b] Bhavan's SPARC, 13th NS Road, JVPD, Juhu, Mumbai 400 049, India
[c] Bio-Organic Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

ARTICLE INFO

Article history:
Received 27 February 2008
Received in revised form 16 April 2008
Accepted 5 May 2008

Keywords:
Ion chromatography
Conductometric detection
Anionic minerals
Black tea
Kombucha tea

ABSTRACT

A simple, rapid and accurate method for the determination of anionic minerals in tea brew has been developed. The quantitative determination of anions – fluoride, chloride, bromide, iodide, nitrate, phosphate and sulphate was accomplished by anion exchange chromatography with conductometric detection. A Metrosep Anion Dual 2 analytical column connected in series with a Metrosep RP guard column was used for anion separation. A solution containing a mixture of 1.3 mM Na_2CO_3 and 2 mM $NaHCO_3$ was used as eluent. The method requires a simple sample clean-up procedure to remove the interfering organic components from the tea brew. The limit of detection for different anionic minerals were in the range 0.01–0.05 μg mL^{-1} and the relative standard deviation were in the range 4–6% for the overall method. The recovery of different anionic minerals added was in the range 95–106%. The method was applied to the determination of anions in black and kombucha tea.

1. Introduction

Tea is one of the most popular beverages in the world. The world annual production of tea is 3.5 million tons. The most popular type of tea in the world is black tea, which is produced in India, Sri Lanka, Kenya and many other countries. Most of the tea produced in Japan is green tea while China produces green, black teas and several other types such as oolong tea, pu-erh tea and kombucha tea. A lot of work has been carried out to explain tea quality in terms of its organic constituents (Finger, Kuhr, & Engelhardt, 1992; Liang, Lu, Zhang, Wu, & Wu, 2003). There are several reports on the determination of cationic minerals in tea (Ferrara, Montesano, & Senatore, 2001; Flaten, 2002; Koch, 1990; Pohl & Prusisz, 2007). However, little work has been done to determine the complete spectrum of anionic minerals in tea. Many anions like fluoride and iodide are very important micronutrients at low concentrations but, at high concentrations, are toxic to the body e.g. an incorrect dietary balance of iodide can lead to thyroid disorders. Excess consumption of fluoride causes symptoms of acute and chronic fluoride toxicity (flourosis).

There are a few reports on the determination of some anionic minerals in tea brew (Alcazar, Fernandez-Caceres, Martin, Pablos, & Gonzalez, 2003; Ding, Chen, & Luo, 1997; Hayacibara, Queiroz, Tabchoury, & Cury, 2004; Prusisz, Grymuza, & Pohl, 2007; Simpson, Shaw, & Smith, 2001; Spiro & Lam, 1995). Michalski (2006) has reported an ion chromatographic method for the simultaneous determination of common inorganic anions in tea infusions. However, it was not possible to determine iodide and bromide content using this method. Furthermore, the detection limits reported in this study were high. Thus none of the methods reported so far are suitable for the quantitative determination of all the anions i.e. F^-, Cl^-, Br^-, I^-, $NO_3{}^-$, $PO_4{}^{-3}$, and $SO_4{}^{-2}$ simultaneously in tea brew. The iodide content of tea brew has not been reported earlier. Hence there is a need to develop a simple, rapid and accurate method for the determination of all the anions in tea.

Ion chromatography technique is being extensively used in food analysis for the simultaneous determination of ions, particularly at trace concentrations (Buldini, Cavalli, & Trifiro, 1997; Fredrikson, Carlsson, Almgren, & Sandberg, 2002; Kumar, Maiti, & Mathur, 2001). The aim of the present study was to use this technique for the determination of trace concentrations of anions in a complex matrix like tea. The presence of large concentration of interfering organic components poses a challenge in the analysis. Hence, in the present work a one step sample pretreatment procedure was adopted to eliminate the matrix interference. Using this procedure it was possible to directly analyze the different anions by ion chromatography in suppressed mode using sodium carbonate–bicarbonate mixture as eluent and conductometric method of detection. The method was applied to the black and kombucha tea.

Kombucha is a traditional fermentation of sweetened tea using a symbiosis of yeast species and acetic acid bacteria (Teoh, Heard,

---

* Corresponding author. Tel.: +91 22 25590322; fax: +91 22 5505151.
E-mail address: sangdk@barc.gov.in (S.D. Kumar).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.012

& Cox, 2004). In recent times kombucha tea is becoming popular due to its therapeutic values (Dipti et al., 2003; Hiremath, Vaidehi, & Mushtari, 2002). It is claimed that it provides overall good health, relief from arthritis-like symptoms and decreases the frequency of infection. As there are no studies reported on the determination of anions in kombucha tea, the present study is first of its kind.

2. Experimental

2.1. Reagents

Black tea (Red Label Brand) was provided by Brooke Bond, Mumbai, India. The kombucha tea was prepared using kombucha culture obtained from Swami Prakasanand Ayurveda Research Centre, SPARC, Mumbai, India. All other reagents were of analytical grade and obtained from Sarabhai M. Chemicals, Baroda, India. The working standard and spiking solutions were prepared by dissolving sodium salt in 18 MΩ deionised water (Barnstead, Boston, MA, USA). On Guard-P and On Guard-RP cartridges were obtained from Dionex (Sunnyvale, CA, USA). On Guard-P has a capacity of 6 meq/cartridge.

2.2. Instrumentation

The ion chromatographic instrument from Metrohm (Herisau, Switzerland) consisting of a 709 IC Pump, 733 IC Separation Centre, a MSM Suppressor with 752 Pump unit and a 732 IC conductivity detector was used for the analysis. Anion separation was carried out in suppressor mode on a Metrosep Anion Dual-2 analytical column (20 mm × 4 mm) connected in series with a Metrosep RP guard column (75 mm × 4.6 mm). A solution containing a mixture of 1.3 mM Na_2CO_3 and 2 mM $NaHCO_3$ flowing at a rate of 0.8 mL min^{-1} served as the eluent. These are the standard operating conditions for the column Metrosep Anion Dual 2 (with chemical suppression). The optimum resolution and elution time for different anions were found under these conditions. A solution of 20 mM H_2SO_4 was used as the regenerant. The volume of the sample injection loop was 20 μL.

Kombucha tea aliquots were centrifuged using a refrigerated centrifuge (R-244, Remi Instruments Ltd., Mumbai, India). The solutions were filtered using sterile bacteria proof filter assembly employing Millex GV filter, 0.22 μm pore size (Millipore, Bedford, MA, USA).

2.3. Procedure

Black tea at a concentration of 0.5% was added to the boiling deionised water. Deionised water was used so that there was no contribution of anions from the water used for preparing the tea brew. It was allowed to seep for 20 min. The decoction was strained through a tea strainer. As the preparation of kombucha tea requires sucrose addition, a sterile concentrated sucrose solution (80%) was added so as to obtain a final concentration of 10%. The black tea brew was allowed to attain room temperature.

A part of the above solution was inoculated with the kombucha culture at a concentration of 10% (w/v) for preparing kombucha tea brew. The inoculated decoction was incubated at 28 ± 2° C for 7 days. The solution was centrifuged for 30 min at 8000 rpm and the obtained supernatant was filtered using a sterile Millipore filter assembly (Bedford, MA, USA).

On Guard-RP cartridge was first washed with 5 mL methanol and then with 10 mL deionised water while the On Guard-P cartridge was washed with 10 mL deionised water. The recommended guidelines for the use of On Guard cartridges supplied by Dionex Corp. (Sunnyvale, CA, USA) were followed. The two cartridges were connected in series with On Guard-P followed by Guard-RP. About 5 mL of the sample solution was loaded on the cartridge and allowed to flow at the rate of 2 mL min^{-1}. The first 3 mL of elute was rejected and the next 2 mL was collected for direct injection into the ion chromatograph. This was done to ensure that there was no dilution of the sample solution during the pretreatment procedure.

2.4. Other analytical method

For comparative purpose, fluoride was determined by fluoride ion-selective electrode (Simpson et al., 2001).

3. Results and discussion

3.1. Interfering organic components in tea and the selection of cartridges

The main characterizing components of tea are its polyphenols often incorrectly referred to as tannins. The polyphenols of black tea constitute 10–20% of its dry weight and 30% of the solids extracted during brewing of the tea to prepare as a beverage. The other important component of tea is caffeine which constitutes 1.5–5% of its dry weight and 5–8% of the solids extracted during brewing (Michels, 2000). Besides this, tea brew is also rich in organic acids e.g. oxalic acid, citric acid, acetic acid etc. (Horie, Yamauchi, & Kohata, 1998). The high quantity of different organic solutes in tea solutions gives rise to several problems like column contamination, poor resolution of inorganic anions, and reduction in sensitivity, etc. In order to overcome these difficulties, a simple and rapid pretreatment procedure was developed using the commercially available On Guard cartridges. The On Guard-P cartridge contains a polyvinyl pyrrolidone (PVP) polymer. It has a very high selectivity for phenolic and other aromatic compounds. The On Guard-RP cartridge contains macroporous divinylbenzene reversed phase packing. Selectivity of this cartridge for hydrocarbons, carboxylic acids and other hydrophobic components is very high. Hence these two cartridges were used for removing the interfering components from the tea brew.

3.2. Preparation of tea brew

The mass of tea leaves to be used for the preparation of tea brew was determined by the concentration of anionic minerals in the sample solutions, so that a measurable signal could be obtained for the different anions. The mass of tea leaves was varied from 0.2–1 g in 100 mL deionised water. It was found that the peak signals for all the anions including bromide and phosphate were measurable at 0.5% concentration of tea brew. Hence, 0.5% tea solution was used for the studies.

3.3. Optimization of sample pretreatment procedure

The criteria for choosing the order in which the two cartridges should be connected in series were governed by the fact that it should enable the identification of all the anion peaks in the sample. It was found that when On Guard-P was placed before the On Guard-RP cartridge, it was possible to identify all the anion peaks in the tea brew (Fig. 1). However, when the sequence was reversed i.e. On Guard-RP placed before the On Guard-P cartridge, it was not possible to identify the fluoride peak in the sample. The reason for this may be that when an On Guard-RP cartridge was used first in the series, the different polyphenols were held at the cost of organic acids, hydrocarbons etc. and on reversing the sequence of cartridges, the polyphenols were first removed selectively followed

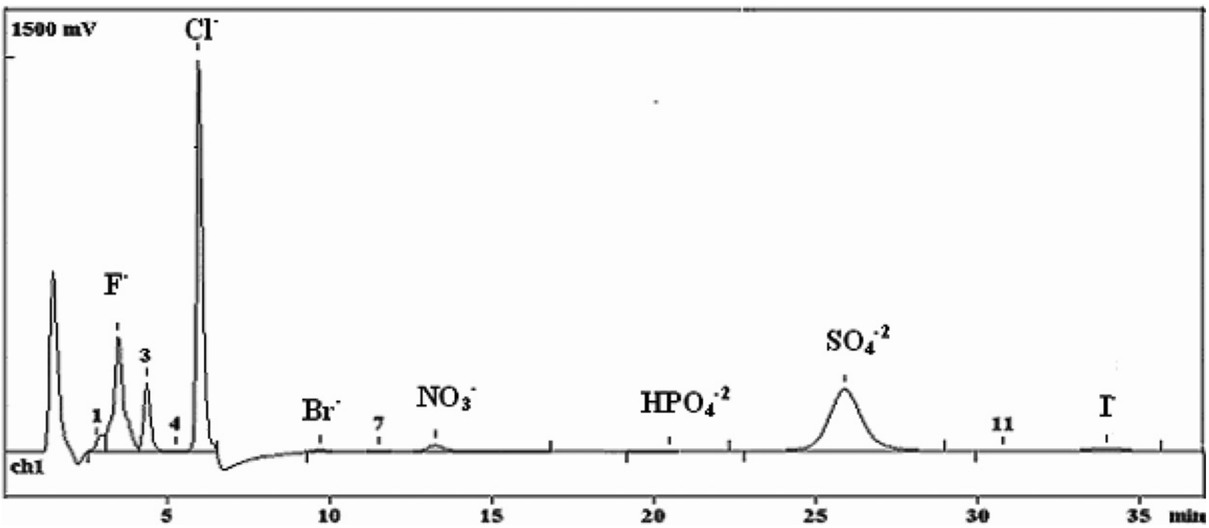

Fig. 1. Chromatogram of the black tea sample solution showing different anionic mineral components peaks using a mixture of 1.3 mM Na_2CO_3 and 2 mM $NaHCO_3$ as eluent, at a flow rate of 0.8 mL min^{-1}.

by organic acids by On Guard-RP. This particular configuration of cartridges also prevented the increase in backpressure of the column. In order to achieve optimum efficiency, the flow rate was maintained as per the recommended guidelines for the cartridges. However, in order to establish this, variation of flow rate was examined and a flow rate of 2 mL min^{-1} was found to be optimum.

3.4. Method validation

The limit of detection LOD ($S/N = 3$), the limit of quantification LOQ ($S/N = 10$) and linear range of calibration for different anionic mineral components are given in Table 1. As shown in Table 1, the calibration graphs of peak areas for all anions are linear over two or three orders of magnitude with a regression coefficient (r^2) of 0.997–0.999. A comparison of the detection limits with those reported by earlier workers showed that 10-fold higher detection limits were obtained by this method. The tea solutions were spiked with different anions concentration (2 μg mL^{-1}) before pretreatment. The recovery of different anionic minerals added was in the range 95–106% (Table 2). Thus, the pretreatment procedure selectively removed the interfering components. The relative standard deviations of different anionic minerals evaluated by replicate analysis ($n = 4$) were <6% (Table 3).

3.5. Anionic minerals in tea samples

The different anionic mineral components identified in black tea were fluoride, chloride, bromide, iodide, nitrate, phosphate and sulphate based on comparisons of tea brew with that of standard anion mixture (Fig. 1). A chromatogram with similar pattern was obtained for kombucha tea (Fig. 2). However, the relative anion concentrations were significantly different as compared to black tea (Table 3). The peaks for the different components except for fluoride were well resolved. In order to identify the fluoride peak, the tea solution was spiked with anion standard solution containing 2 μg mL^{-1} prior to pretreatment. The peaks co-eluting with fluoride may be due to acetate and formate ions because of their close retention times with fluoride ion. The dip appearing just after the chloride peak was due to the eluent anions (carbonate) and called the system peak (Schafer, Laubil, & Dorig, 2003). The nitrate and bromide peaks were also identified in the brew and hence are reported for the first time in this paper (Table 3). It is clear from the chromatogram that the iodide has a very high affinity for the anion exchanger and hence was eluted out last from the column (retention time $t_r = 35$ min). It is conjectured that it could be one of the reasons why nobody has reported iodide concentration in tea.

Table 2
Recovery studies of anionic minerals in the tea brew using On Guard-P and On Guard-RP cartridges

Anion	Concentration (μg mL^{-1})		Recovery (%)
	Added	Found	
F^-	2.0	2.10	105
Cl^-	2.0	1.98	99
Br^-	2.0	2.12	106
NO_3^-	2.0	1.90	95
HPO_4^{-2}	2.0	1.95	97.5
SO_4^{-2}	2.0	2.10	105
I^-	2.0	1.94	97

Table 1
Different parameters obtained using standard anion mixture solutions

Anion	Linearity range (μg mL^{-1})	r^2	LOQ (μg mL^{-1})	LOD (μg mL^{-1})		
				Present	Ding et al. (1997)	Alcazar et al. (2003)
F^-	0.03–100	0.997	0.03	0.01	–	–
Cl^-	0.03–100	0.999	0.03	0.01	0.04	0.6
Br^-	0.03–100	0.997	0.06	0.02	0.14	–
NO_3^-	0.03–100	0.999	0.06	0.02	0.11	–
HPO_4^{-2}	0.03–100	0.998	0.06	0.02	0.12	4.1
SO_4^{-2}	0.03–100	0.999	0.06	0.02	0.19	–
I^-	0.20–100	0.998	0.17	0.05	–	–

Table 3
Anionic mineral concentrations of different tea (in brackets the values obtained using ion-selective electrode are given)

Anion mg g^{-1}	KT^a	BT^b	BT^a	GT^c	BT^a	OT^d	JasT^e	GT^c	JapT^f
	Present	Present	Spiro et al. (1995)		Alcazar et al. (2003)		Ding et al. (1997)		
F^-	3.20 ± 0.16 (3.12)	1.20 ± 0.06 (1.28)	0.06	0.08	–	–	–	–	–
Cl^-	0.96 ± 0.04	3.12 ± 0.13	0.90	0.53	0.60	0.55	1.04	1.78	1.94
Br^-	0.04 ± 0.01	0.04 ± 0.01	–	–	–	–	–	–	–
NO_3^-	0.18 ± 0.01	0.34 ± 0.02	–	–	–	–	–	–	–
HPO_4^{-2}	0.04 ± 0.01	0.08 ± 0.01	1.18	0.90	2.93	2.34	8.00	7.88	11.84
SO_4^{-2}	1.02 ± 0.04	4.20 ± 0.17	1.45	2.13	–	–	3.58	4.58	1.54
I^-	1.04 ± 0.08	0.44 ± 0.04	–	–	–	–	–	–	–

The results are means ± standard deviation ($n = 4$).

[a] Kombucha tea.

[b] Black tea.

[c] Green tea.

[d] Oolong tea.

[e] JasT – Jasmine tea.

[f] JapT– Japanese tea.

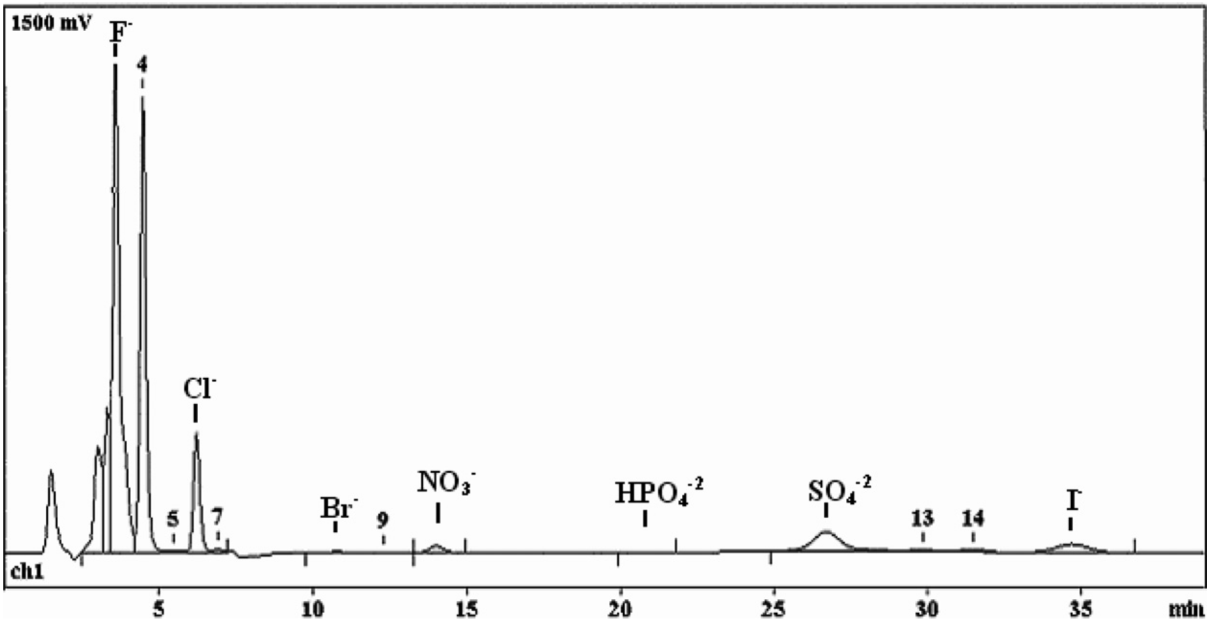

Fig. 2. Chromatogram of the kombucha tea sample solution showing different anionic mineral components peaks using a mixture of 1.3 mM Na_2CO_3 and 2 mM $NaHCO_3$ as eluent, at a flow rate of 0.8 mL min^{-1}.

Table 3 presents the results of complete spectrum of mineral anions obtained for black and kombucha tea samples. These have been compared with the few available in the literature. The chloride and sulphate contents of black tea are in good agreement with the values obtained by earlier workers (Alcazar et al., 2003; Ding et al., 1997; Spiro et al., 1995). But there is a significant difference between the fluoride and phosphate content. Measuring the anionic mineral concentration in the tea samples using ion-selective electrodes was attempted. However, due to inherent limitations of the ion-selective electrode method only fluoride ion could be determined. The values obtained were in close proximity (Table 3). The measurement of other anions (Cl^-, Br^-, I^-, and NO_3^-) using ion-selective electrodes was not successful due to interference from the matrix elements.

4. Conclusions

The present study was attained to develop a simple, accurate and rapid analytical methodology for the simultaneous determination of – fluoride, chloride, bromide, iodide, nitrate, phosphate and sulphate in the tea brew. The proposed method has several advantages over other methods. It is possible to determine all the anionic minerals simultaneously with ten fold lower detection limits and improved resolution. The studies have revealed that black and kombucha tea differ significantly in their anionic mineral composition.

References

Alcazar, A., Fernandez-Caceres, P. L., Martin, M. J., Pablos, F., & Gonzalez, A. G. (2003). Ion chromatographic determination of some organic acids, chloride and phosphate in coffee and tea. *Talanta, 61*, 95–101.

Buldini, P. L., Cavalli, S., & Trifiro, A. (1997). State-of-the-art ion chromatographic determination of inorganic ions in food. *Journal of Chromatography A, 789*, 529–548.

Dipti, P., Yogesh, B., Kain, A. K., Pauline, T., Anju, B., Sairam, M., et al. (2003). Lead induced oxidative stress: Beneficial effects of kombucha tea. *Biomedical and Environmental Sciences, 16*, 276–282.

Ding, M. Y., Chen, P. R., & Luo, G. A. (1997). Simultaneous determination of organic acids and inorganic anions in tea by ion chromatography. *Journal of Chromatography A, 764*, 341–345.

Ferrara, L., Montesano, D., & Senatore, A. (2001). The distribution of minerals and flavonoids in the tea plant (*Camellia sinensis*). *Il Fármaco, 56*, 397–401.

Finger, A., Kuhr, S., & Engelhardt, U. H. (1992). Chromatography of tea constituents. *Journal of Chromatography A, 624*, 293–315.

Flaten, T. P. (2002). Aluminium in tea/concentrations, speciation and bioavailability. *Coordination Chemistry Reviews, 228*, 385–395.

Fredrikson, M., Carlsson, N. G., Almgren, A., & Sandberg, A. S. (2002). Simultaneous and sensitive analysis of Cu, Ni, Zn, Co, Mn, and Fe in food and biological samples by ion chromatography. *Journal of Agricultural and Food Chemistry, 50*, 59–65.

Hayacibara, M. F., Queiroz, C. S., Tabchoury, C. P. M., & Cury, J. A. (2004). Fluoride and aluminum in teas and tea-based beverages. *Revista de Saúde Pública, 38*, 100–106.

Hiremath, U. S., Vaidehi, M. P., & Mushtari, B. J. (2002). Effect of Fermented tea on the blood sugar levels of NIDDM subjects. *The Indian Practitioner, 55*, 423–425.

Horie, H., Yamauchi, Y., & Kohata, K. (1998). Analysis of organic anions in tea infusions using capillary electrophoresis. *Journal of Chromatography A, 817*, 139–144.

Koch, K. R. (1990). Quantitative determination of aluminium in tea by means of aluminium-27 nuclear magnetic resonance spectroscopy. *Analyst, 115*, 823–825.

Kumar, S. D., Maiti, B., & Mathur, P. K. (2001). Determination of iodate and sulphate in iodized common salt by ion chromatography with conductivity detection. *Talanta, 53*, 701–705.

Liang, Y., Lu, J., Zhang, L., Wu, S., & Wu, Y. (2003). Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. *Food Chemistry, 80*, 283–290.

Michels, M. J. M. (2000). Teas, herbal teas, and coffee. In B. M. Lund, T. C. Baird-Parker, & G. W. Gould (Eds.), *The microbiological safety and quality of food* (Vol. 1, pp. 960–961). Maryland: Aspen Publishers.

Michalski, R. (2006). Simultaneous determination of common inorganic anions in black and herbal tea by suppressed ion chromatography. *Journal of Food Quality, 29*, 607–616.

Pohl, P., & Prusisz, B. (2007). Fractionation analysis of manganese and zinc in tea infusions by two-column solid phase extraction and flame atomic absorption spectrometry. *Food Chemistry, 102*, 1415–1424.

Prusisz, B., Grymuza, K., & Pohl, P. (2007). Interferences elimination in ion chromatography by inclusion of the interfering ion into the mobile phase—Analysis of fluoride in chloride rich dietary samples. *Talanta, 71*, 1364–1368.

Schafer, H., Laubil, M., & Dorig, R. (2003). Ion chromatography. *Metrohm Monograph, 50143*, 11.

Simpson, A., Shaw, L., & Smith, A. J. (2001). The bio-availability of fluoride from black tea. *Journal of Dentistry, 29*, 15–21.

Spiro, M., & Lam, P.-L. L. (1995). Kinetics and equilibria of tea infusion—Part 12. Equilibrium and kinetic study of mineral ion extraction from black Assam Bukial and green Chun Mee teas. *Food Chemistry, 54*, 393–396.

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of kombucha fermentation. *International Journal of Food Microbiology, 95*, 119–126.