Determination of Acidity of Coffee by Flow Injection Analysis with Electrochemical Detection

Tetsuo Fuse, Fumiyo Kusu, and Kiyoko Takamura*

School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

The present study was conducted to establish a sensitive, rapid, reliable, and accurate method for determining the acidity of coffee by flow injection analysis (FIA) using an electrochemical detector with a glassy carbon electrode and a carrier solution of ethanol containing 38 mM $LiClO_4$ and 3 mM 2-methyl-1,4-naphthoquinone (vitamin K_3, VK_3). FIA signals for 21 coffee samples showed good correlation with titratable acidity, a common chemical measure of the sourness of coffee. FIA signals were also related with sourness intensity determined by sensory panel test. Change in the acidity of coffee, due to roasting of coffee beans, was monitored by FIA. The present method was found adequate for determining coffee acidity.

Keywords: *Coffee; acidity; titratable acidity; electrochemical detection*

INTRODUCTION

Various acids, such as chlorogenic acid, citric acid, and acetic acid, are present in coffee beans. The origin and growth conditions of the coffee plant are often the causes for considerable differences in acid content. Acid content differs according to the kind of coffee bean and changes easily by the mode of processing, roasting, and extraction. Slight change in acid content readily results in differences in taste and aroma of coffee. Acid assay of coffee beans and coffee is thus important for quality control. Sensory tests are usually conducted for quality assessment but are not adequate for lack of objectivity and efficiency.

Common methods of acid assay, involving alkali titration and pH measurement (Suzuki, 1978), are not sufficiently sensitive to follow slight acidity changes. Gas chromatography (GC) (Engelhardt and Maier, 1985a,b; Wada et al., 1987), high-performance liquid chromatography with UV detection (HPLC/UV) (Badoud and Pratz, 1986; Schwarzenbach, 1982), and capillary electrophoresis (Weers et al., 1995) are used to determine carboxylic acids in coffee beans or coffee, but these methods are not always suitable for routine work. In general, GC is used only for volatile components. In HPLC/UV, owing to poor light absorptivity of the carboxyl group, complicated derivatization of acids with chromophores is sometimes required. For determination by capillary electrophoresis, acids should be chromophores. A more sensitive, simple, and rapid method is thus desirable for acid assay in quality control of coffee beans and coffee.

The authors previously developed a new flow injection analysis (FIA) method for determining free fatty acids in oils, based on the fact that trace amounts of acid present in an ethanol solution of 2-methyl-1,4-naphthoquinone (vitamin K_3, VK_3) cause a prepeak on the voltammogram prior to the normal reduction peak of VK_3 (Takamura et al., 1995). Total amounts of free fatty acids in samples were determined from the prepeak height (Kusu et al., 1994; Fuse et al., 1995). Electrochemical detection (FIA/ECD) was confirmed useful for determining acid content in oils (Takamura et al., 1995).

* Corresponding author (fax +81-426-76-4570; e-mail takamura@ps.ac.jp).

In this paper we have studied the possibility to use the same methodology for acid assay of coffee.

MATERIALS AND METHODS

Reagents. Chlorogenic acid (3-caffeoylquinic acid) (96%), caffeic acid (98%), and quinic acid (98%) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and used without further purification. Other chemicals used were of reagent grade and obtained from a commercial source (Wako Pure Chemical Industries, Osaka, Japan). Pure water was obtained using the NANOpure II purification system (Hansen & Co., Ltd., Kobe, Japan). Ethanol solution containing 3 mM VK_3 and 38 mM $LiClO_4$ served as the carrier solution for FIA. A mixture of acetonitrile−10 mM phosphoric acid (25:75) was used as a mobile phase for HPLC.

Sample Preparation. For the coffee preparation, 250 mL of hot water was poured onto 20 g of coffee powder on a filter paper, using a filter (Kalita model EX-102, Tokyo, Japan).

Coffee from 21 different coffee powders commercially available from UCC Ueshima Coffee Ltd. (Kobe, Japan), Key Coffee Co., Ltd. (Tokyo, Japan), and Ajinomoto Co., Inc., (Tokyo, Japan) were used for the determination of acidity by FIA and titration and the sensory test by panelists. Roasted coffee beans were purchased from a coffee grain shop (Green Bean, Hachioji, Japan), the shop was asked to roast green coffee beans [Kilimanjaro (Tanzania), Guatemala (Guatemala), and Hawaii Kona (Hawaii)] by hot air heating for 0−20 min. The beans were subsequently powdered with a coffee mill (National model MK-52M, Tokyo, Japan) at our laboratory.

Apparatus. The flow injection system consisted of a pump model DMX-2200-T, SNK Ind. Co. Ltd. (Tokyo, Japan), sample injector model 7125, Rheodyne Inc. (Cotati, CA), electrochemical cell, potentiostat model 312, Huso Electro Chemical System (Kawasaki, Japan), and a recorder model 807-IT, Jasco Co. (Tokyo, Japan). The electrochemical cell was fabricated from a glassy carbon, Tokai Carbon Co., diameter 6 mm (Tokyo, Japan), working electrode, an Ag/AgCl reference electrode, a stainless-steel auxiliary electrode, and a polychlorotrifluoroethylene cell body. The cell volume was 2.4 μL (Takamura et al., 1995). Titration was conducted using an automatic potentiometric titrator model AUT-301, TOA Electronics Ltd. (Tokyo, Japan). The HPLC system consisted of a pump model 880-PU, Jasco Co. (Tokyo, Japan), LiChrospher column 100 RP-18, 250 mm × 4 mm i.d., 5 μm, Cica-Merck (Tokyo, Japan), sample injector model 7125, Rheodyne Inc. (Cotati, CA), and multiwavelength detector model MD-910, Jasco Co. (Tokyo, Japan).

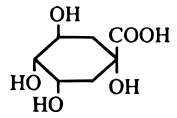
Procedure. For FIA measurement, coffee was diluted with ethanol solution containing 3 mM VK_3 and 38 mM $LiClO_4$ to

S0021-8561(96)00690-5 CCC: $14.00

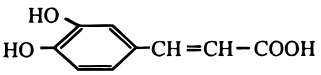
Table 1. Typical Acids Present in Coffee

formic acid:	HCOOH
acetic acid:	CH_3COOH
fumaric acid:	HOOCCHCHCOOH
malic acid:	$HOOCCHOHCH_2COOH$
citric acid:	$HOOCCH_2C(OH)(COOH)CH_2COOH$
higher fatty acid:	$CH_3(CH_2)_nCOOH$

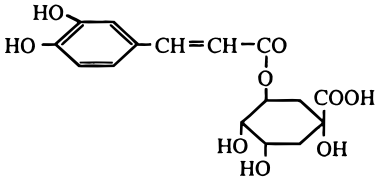
quinic acid:



caffeic acid:



chlorogenic acid:



prepare the test solution. The detection potential for monitoring acid content was maintained at −0.33 V vs Ag/AgCl. Acid content in the test solution was determined based on peak height of flow signals. The flow rate of the carrier solution was 0.6 mL/min. A 5 μL test solution was injected into the flow injection system.

To determine titratable acidity, 50 mL of coffee was neutralized with 0.100 M NaOH until pH 8.2 (Suzuki, 1978) using the automatic potentiometric titrator. The volume of 0.100 M NaOH required was defined as titratable acidity.

For the sour sensory test, seventeen students and staff at the Tokyo University of Pharmacy and Life Science in the Laboratory of Analytical Chemistry were available. A tray of randomly coded hot coffees, a sensory test ballot of a 5-point linear scale, and a cup of water were used for evaluating. Each coffee was assessed for sourness intensity using the 5-point scale from 1 to 5 (scoring test). Mean scores were obtained from the data.

RESULTS AND DISCUSSION

Acid Determination by Means of Electrochemical Reduction of Quinone. In general, quinone gave a clear reduction peak on the voltammogram at its reduction potential. In previous studies on the electrochemical reduction of quinone, small amounts of acids in a quinone solution were found to give rise to a prepeak on the voltammogram at a potential less negative than for the reduction peak of quinone (Takamura and Hayakawa, 1971). This prepeak was found due to the protonation of quinone at the electrode surface prior to its electron transfer. Protonated quinone is reduced at a potential less negative than that of quinone. Prepeak height was proportional to acid concentration and the half-peak potential of the prepeak shifted to a more negative potential accompanied by increase in pK_a of the acid. If a weak acid was almost undissociated in a quinone solution, the dissociation was accelerated at the electrode surface by the protonation of quinone and consumption of the protonated quinone. Total acid in the solution could thus be determined at an applied potential that would give the reduction current of protonated quinone. The present authors have used these findings in FIA/ECD to determine free fatty acid content in oils, and sensitivity, reproducibility, and rapidity were found far superior to conventional titration method using KOH (Takamura et al., 1995).

FIA/ECD was thus applied to determine acid content in coffee. VK_3 was the quinone species since its solubility and stability in the present medium would facilitate conducting the assay. Measurement of flow signals at various concentrations of chlorogenic acid, a major constituent acid in coffee (Table 1), was initially conducted. As shown in Figure 1, a flow signal appeared at each concentration of chlorogenic acid. The response was linear between 5.0×10^{-6} and 3.0×10^{-4} M. Chlorogenic acid at 1.0×10^{-4} M was determined 10 times with a relative standard deviation (RSD) of 1.4%. There was no carry over between samples even at a rate of 40 samples per hour as the signal still reached the baseline. Linearity and reproducibility were basically the same for other acids listed in Table 1. The detection system was stable for about six months when processing acid solutions or diluted coffee samples.

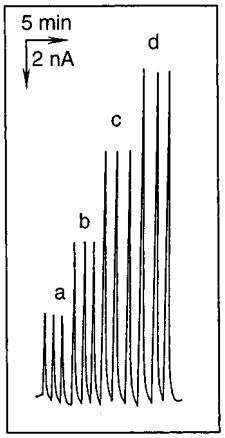


Figure 1. FIA signals for (a) 5.0×10^{-5}, (b) 1.0×10^{-4}, (c) 1.5×10^{-4}, (d) 2.0×10^{-4} M chlorogenic acid. Applied potential, −330 mV vs Ag/AgCl: injection volume of sample solution, 5 μL: flow rate, 0.6 mL/min.

Coffee contains various acids having one or more acid functional groups and different acid strengths. The slope of the FIA signal current versus concentration depends on the type of acid (Kusu et al., 1995). Thus the FIA signal, I, is given by

$$I = \sum_i k_i C_i \qquad (1)$$

where C_i is the total concentration of acid i and k_i the proportionality constant of an individual FIA signal current to be related to the concentration of acid i.

Titration of Chlorogenic Acid. Coffee beans contain various acid components, some of which are listed in Table 1 (Clifford, 1975; Weers et al., 1995). The content of any acid in coffee is small, but the total amount of acids has a significant effect on the sourness and aroma of coffee. Sour taste is correlated to total amounts of acids in coffee. Thus, titratable acidity is a measure of sourness in chemical analysis.

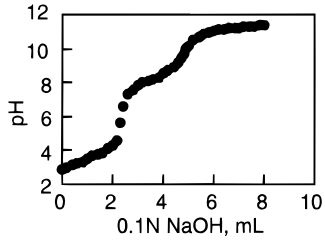


Figure 2. Titration of 50.0 mL of 4.6 mM chlorogenic acid with 0.100 M NaOH.

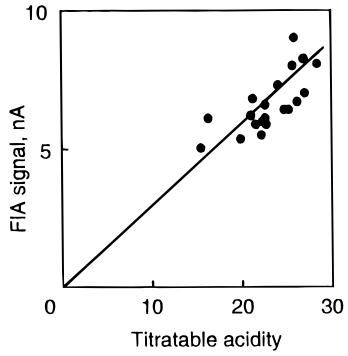


Figure 3. Correlation of FIA signal and titratable acidity for the 21 coffees.

Figure 2 shows the titration curve for 50 mL of 4.6 mM chlorogenic acid. Two end points appeared on the titration curve. The first and second end points seem to be due to titration of the carboxylic acid group and the phenolic hydroxyl groups of chlorogenic acid, respectively. The first inflection corresponded to the equivalence point, i.e., 2.3 mL of 0.100 M NaOH. When pH of the end point was 8.2 in determining titratable acidity, 50% excess of 0.100 M NaOH was required. Chlorogenic acid determination by FIA was found accurate because the volume of 0.100M NaOH corresponding to the amount of acid determined agreed with the first end point in Figure 2.

Caffeic acid as well as chlorogenic acid was present in the solution mixture of chlorogenic acid and NaOH obtained at the titration just before the second jump. This fact was confirmed by HPLC with UV detection at 332 nm and TLC. Although quinic acid could not examined because of its non-absorption property, about 10% of chlorogenic acid was considered to be saponified in alkaline solution to produce caffeic and quinic acids during titration. Coffee beans contain chlorogenic acid homologs at several percent, as mono- or diesters of cinnamic and quinic acids, and thus qualitative and quantitative changes of chlorogenic acid homologs may occur easily when coffee beans are roasted. Chlorogenic acid homologs should thus be determined correctly to measure the sourness of coffee. Determination of titratable acidity by titration using alkaline conditions thus would not be an ideal method to quantify total amounts of acids in coffee.

Application to Acid Assay in Coffee. Although titration has the problem described above, titratable acidity commonly serves as a measure for evaluating the sourness of coffee. Comparison was thus made of FIA signals with titratable acidity for coffee using purchased coffee powder.

The FIA signal showed good correlation ($r = 0.814$) with the titratable acidity of 21 coffee samples (Figure 3), suggesting this parameter to be an alternate means for coffee quality assessment.

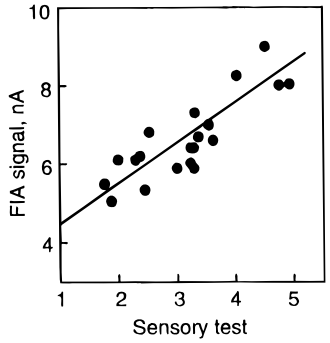


Figure 4. Relation between FIA signal and sourness intensity scores.

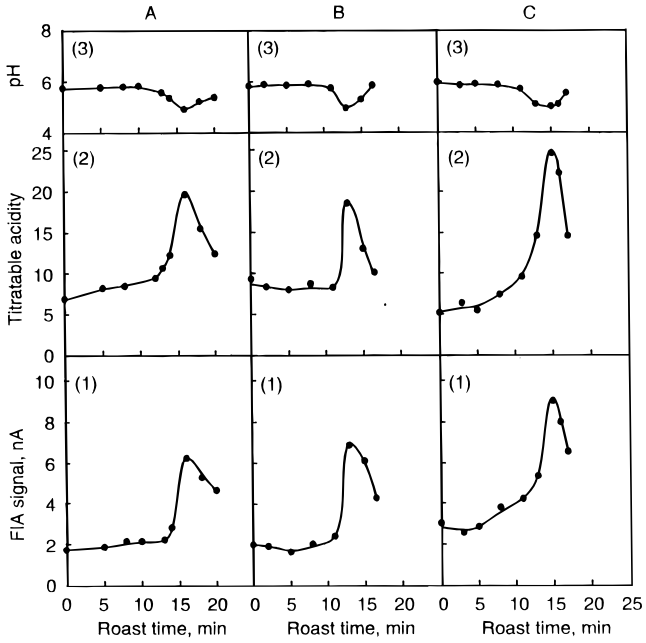


Figure 5. Effects of roasting time on acidity of coffee measured by FIA (1), titration (2), and pH meter (3) with coffee beans (A) Kilimanjaro, (B) Guatemala, and (C) Hawaii Kona.

Sour taste is considered to reflect the number of acid molecules that come into contact with gustatory buds on the tongue surface. In the FIA/ECD method, the current signal corresponds to acid amount supplied to the working electrode surface. The present method should thus prove useful for assessing coffee sourness. This was found so by a comparison of FIA signals with sourness sensory test data. The 5-point sensory test for sourness intensity was made for 21 coffees prepared using commercially available coffee powders. Mean scores were compared to FIA signals and good correlation ($r = 0.847$) was noted, as shown in Figure 4.

From Figures 3 and 4, the present method, as well as titratable acidity measurement, appear reliable means for assessing sour taste. pH values for roasted Kilimanjaro (K), Guatemala (G), and Hawaii Kona (H) was basically the same, from 4.9 to 5.0. On the other hand, even the test solution for the present method was highly diluted compared to those for titration and pH measurement, FIA revealed a distinct current signal of 6.2−9.0 nA.

Sour taste is determined in part by the mode of roasting. Acid content attains a maximum and then decreases with roasting time. Change in sour taste was examined during roasting. FIA signal current was plotted against roasting time. As seen in Figure 5, B(1), current remained essentially constant and low up to 11

min, then increased markedly and finally attained a maximum at about 13 min, and then decreased gradually. Sensory test results from 7 participants for the same coffee as above clearly showed acid taste intensity to change with the roasting time in the order, 13 > 15 > 16.5 > 0 min. Comparison of the results showed excellent agreement, suggesting the current of FIA signal serves as an accurate indication of sour taste.

Change in titratable acidity and pH of coffees extracted from K, G, and H coffee beans was also followed during roasting, and the results are shown in Figure 5, A−C, as (2) and (3), respectively. A pH vs time curve showed a minimum value at 13−16 min [Figure 5, (3)]. In the pH measurement, only a slight change was noted throughout the roasting period in contrast to Figure 5, (1), since the potential of the glass electrode cell responds only to the logarithm of the concentration of dissociated protons in the sample solution. In Figure 5, (2), titratable acidity is plotted against roasting time to give a curve similar to that in Figure 5, (1), which is as expected from Figure 3. Titration until pH 8.2 may be attended with small error due to end point mismatch, not to mention the large amount of sample and time required: 50 mL of coffee and about 10 min for each plot in Figure 5, (2). Only 5 μL of 40-fold diluted coffee and about 1.5 min are required for each plot in Figure 5, (1).

Coffee bean quality is characterized not only by the origin but also by the growth stage and varies strikingly according to the mode of roasting prior to use for coffee beverage. Acid taste cannot be assessed only on the basis of bean appearance. A reliable method for evaluating taste appears to be provided by the present FIA/ECD.

LITERATURE CITED

Badoud, R.; Pratz, G. Improved high-performance liquid chromatographic analysis of some carboxylic acids in food and beverages as their *p*-nitrobenzyl esters. *J. Chromatogr.* **1986**, *360*, 119−136.

Clifford, M. N. The composition of green and roasted coffee beans. *Process Biochem.* **1975**, *5*, 13−16.

Engelhardt, U. H.; Maier, H. G. Determination of non-volatile acids in coffee: comparison of capillary isotachophoresis and capillary gas chromatography. *Fresenius Z. Anal. Chem.* **1985a**, *320*, 169−174.

Engelhardt, U. H.; Maier, H. G. Acids of coffee. XII. Contribution of individual acids to the sour taste. *Z. Lebensm. Unters. Forsch.* **1985b**, *181*, 206−209.

Fuse, T.; Kusu, F.; Takamura, K. Voltammetric determination of free fatty acid content in fats and oils. *Bunseki Kagaku* **1995**, *44*, 29−33.

Kusu, F.; Fuse, T.; Takamura, K. Voltammetric determination of acid values of fats and oils. *J. AOAC Int.* **1994**, *77*, 1686−1689.

Kusu, F.; Fuse, T.; Takamura, K. Determination of acid content in coffee beans and coffee. *Colloq. Sci. Int. Cafe, [C.R.], 16th* **1995**, *1*, 351−358.

Schwarzenbach, R. High-performance liquid chromatography of carboxylic acids. *J. Chromatogr.* **1982**, *251*, 339−358.

Suzuki, N. *Japanese Standard Methods for the Examination of Foods*; Japan Food Hygiene Association: Tokyo, 1978; Chapter 23.

Takamura, K.; Fuse, T.; Kusu, F. Determination of the free fatty acid content in fats and oils by flow injection analysis with electrochemical detection. *Anal. Sci.* **1995**, *11*, 979−982.

Takamura, K.; Hayakawa, Y. Effects of proton donors on the polarographic reduction of methyl-*p*-benzoquinone in aqueous and methyl cellosolve solution. *J. Electroanal. Chem.* **1971**, *31*, 225−232.

Wada, K.; Sasaki, H.; Shimoda, M.; Osajima, Y. Studies on aroma of coffee. Part IX. Objective evaluation of various trade varieties of coffee by coupling of analytical data and multivariate analyses. *Agric. Biol. Chem.* **1987**, *51*, 1753−1760.

Weers, M.; Balzer, H.; Bradbury, A.; Vitzthum, O. G. Analysis of acids in coffee by capillary electrophoresis. *Colloq. Int. Sci. Cafe, [C.R.], 16th* **1995**, *1*, 218−223.

Received for review September 12, 1996. Revised manuscript received February 20, 1997. Accepted March 5, 1997.[⊗]

JF960690J

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.