

A Prototype Portable Voltammetric Sensor for Determining Titratable Acidity of Sake and Moromi

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The total content of acidic compounds in sake is called titratable acidity, which is a parameter to evaluate the taste of sake. Thus, the titratable acidity of sake and moromi (sake mash) is frequently determined for their control during brewing. In this study, a portable sensor has been developed for determining the titratable acidity of sake and moromi based on the voltammetric measurements of the reduction prepeak of 3,5-di-*tert*-butyl-1,2-benzoquinone (DBBQ) in the presence of acidic compounds. The portable sensor, which consists of a prototype potentiostat, a function generator, a mobile lithium-ion battery, a recorder, and an electrochemical cell, was used to measure a voltammogram of DBBQ. The prepeak currents of DBBQ were proportional to the concentrations of succinic acid ranging from 1.33 to 33.3 mM, and thus the portable sensor could be applied to determine titratable acidity ranging from 0.266 to 6.66. In real sample analyses using commercially available sakes and moromi samples fractionated during brewing, the titratable acidities determined using the portable sensor were essentially the same compared with those determined by neutralization titration. These results demonstrate that our portable sensor is useful for on-site analysis for the quality control of sake.

1. Introduction

Organic acids such as succinic, citric, and lactic acids in sake (traditional Japanese rice wine) have been reported to play significant roles in the taste and flavor.^(1–3) The total content of acidic compounds in sake is called titratable acidity, and it is a parameter that represents the taste of sake. Thus, titratable acidity is a parameter to be controlled during the brewing process of sake and/or to refer to when selecting a sake for personal consumption. Titratable acidity of sake is defined as the volume (mL) of 0.1 M sodium hydroxide solution required to neutralize acidic compounds in 10 mL of sake.^(4,5) To determine the titratable acidity of sake, neutralization titration, which is used to determine the endpoint from the color change of mixture indicators (bromothymol blue and neutral red) or the measured pH (up to pH at 7.2), has been adopted as the official method of the National Tax Agency Japan.^(4,5) Although this neutralization titration

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is precise, it requires a large sample volume and much measurement time for the assay. An analytical method such as using a sensor, which can save on sample volume, reduce measurement time, and be applied to on-site analysis, is desirable for both brewing companies and personal use. Because an electrochemical analysis such as voltammetry offers attractive features including easy miniaturization, rapid analysis speed, low cost, and low power consumption,^(6–9) it could resolve the above-mentioned issues. Thus, the development of an electrochemical sensor for determining the titratable acidity of sake and moromi will be of value to the brewing industry. However, the titratable acidity of sake cannot be determined by common voltammetry because the main acidic compounds, such as succinic, citric, and lactic acids are electro-inactive.^(10–13)

Previously, an electrochemical detection system was developed for determining electro-inactive acidic compounds on the basis of the voltammetric reduction of 3,5-di-*tert*-butyl-1,2-benzoquinone (DBBQ).⁽¹⁴⁾ The electrode redox process of DBBQ involves a one electron transfer coupled with a one proton transfer from a protic solvent such as ethanol. The presence of a small amount of acidic compound in an unbuffered protic solvent containing DBBQ was found to cause a new peak (termed a prepeak) at a more positive potential than the original reduction peak of DBBQ itself. The peak current height of the prepeak was found to increase with the increase in the amount of acidic compound in a manner proportional to the concentration of the acidic compound, giving a basis for the voltammetric detection of acidic compounds. On the basis of this finding, a voltammetric sensor was provided for determining the titratable acidity of *Schisandrae Fructus*⁽¹⁴⁾ and sake,⁽¹⁵⁾ i.e., the reported voltammetric sensor was provided for determining only weak acids. In another study regarding the analysis of sake, a prototype sensor using a prototype potentiostat and a mobile lithium-ion battery was developed for determining amino acidity, which is another parameter in the taste of sake.⁽¹⁶⁾ The measurement principle of the voltammetric sensing of amino acid is based on a concept of acid-base back titration and a decrease in the reduction prepeak current of DBBQ caused by surplus HCl, which is obtained by the neutralization between excess HCl and amino acids in sake.⁽¹⁶⁾ Thus, the prototype sensor determines a strong acid such as HCl. Another behavior of the reduction prepeak of DBBQ is that the potential of the prepeak becomes more positive with the increase in the acid strength of the compounds, i.e., the prepeak of DBBQ caused by a strong acid shows a more positive potential than that caused by a weak acid. The prototype sensor⁽¹⁶⁾ can arbitrarily set a potential sweep range, whereas the reported voltammetric sensor^(14,15) cannot do it, so the reported voltammetric sensor^(14,15) cannot perform the determination of amino acidity of sake. If both the amino acidity and titratable acidity of sake can be determined using a single sensor, such a sensor would be meaningful for brewing companies and sake lovers alike.

In this study, a portable sensor has been developed for determining the titratable acidity of sake and moromi (sake mash) by means of the voltammetric reduction of DBBQ. By real sample analyses using commercially available sakes and moromi samples fractionated during brewing, our portable sensor was proven to have sufficient accuracy and precision for determining the titratable acidity of sake and moromi.

2. Materials and Methods

2.1 Portable sensor

As shown in Fig. 1, the portable sensor consists of a prototype potentiostat [13(W) × 9(D) × 6(H) cm, TOPPAN, Tokyo, Japan], a function generator (AWG-10K, ELMOS, Osaka, Japan), a mobile lithium-ion battery (CHE-059, TRA, Osaka, Japan), a recorder (GL-100, GRAPHTEC, Kanagawa, Japan), and an electrochemical cell. A glassy carbon working electrode (ϕ 3 mm, disk, BAS, Japan), an Ag/AgCl reference electrode, and a coiled Pt wire counter electrode (ϕ 0.5 mm × 50 mm length) were inserted through the lid into the electrochemical cell. Linear sweep voltammetry was started at a potential of +0.4 V vs Ag/AgCl, and voltammograms were recorded at a scan rate of 20 mV/s.

2.2 Materials and chemicals

Four types of commercially available sake (labeled A, B, C, and D) were obtained from a supermarket in western Tokyo. The moromi samples were supplied from the brewing company that produces sake (A). DBBQ (>98%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Ethanol (>99.5%), isopropanol (>99.7%), sodium chloride (>99.5%), succinic acid (>99.5%), acetic acid (>99.7%), and a 0.1 M sodium hydroxide solution were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan).

2.3 Preparation of test solution for voltammetry

To prepare the test solution for voltammetry, 0.3 mL of sake, moromi, or succinic acid standard solution was added to 3.0 mL of a DBBQ cocktail⁽¹⁵⁾ consisting of an ethanol–water–isopropanol (5:4:1, v/v/v) mixture containing 0.1 M sodium chloride, 15 mM DBBQ, and 1.5 mM acetic acid. After this, the test solution was transferred to an electrochemical cell.

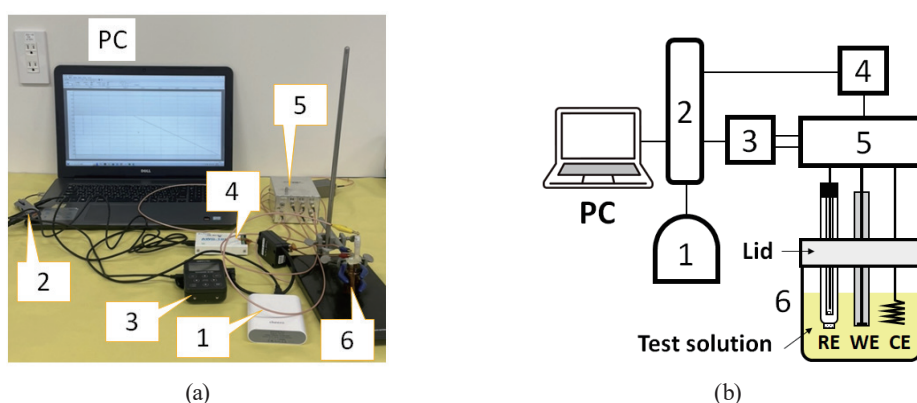


Fig. 1. (Color online) (a) Photograph and (b) schematic diagram of the portable sensor. 1: Mobile lithium-ion battery; 2: multiple USB ports; 3: recorder; 4: function generator; 5: prototype potentiostat; 6: electrochemical cell. RE: reference electrode; WE: working electrode; CE: counter electrode; PC: notebook computer.

2.4 Neutralization titration

Neutralization titration for determining the titratable acidity of sake and moromi was performed using an automatic potentiometric titrator (AUT-701, DKK-TOA, Tokyo, Japan) following the official method of the National Tax Agency Japan.⁽⁴⁾ Ten milliliters of sake or the moromi sample was titrated with a 0.1 M sodium hydroxide solution up to an endpoint of pH 7.2.

3. Results and Discussion

3.1 Voltammetric behaviors of DBBQ in the presence of succinic acid

First, an ethanol–water–isopropanol (5:4:1, v/v/v) mixture containing 0.1 M sodium chloride, 15 mM DBBQ, and 1.5 mM acetic acid was determined to be the optimal DBBQ cocktail to be used for determining titratable acidity for the following reasons: (a) the ethanol–water–isopropanol (5:4:1, v/v/v) mixture easily dissolved DBBQ and sodium chloride, which was used as a low-cost supporting electrolyte, (b) the mixture also dissolved the components in sake, (c) the next assay can be performed after rinsing the electrodes with 70% ethanol because the components in sake and the electrolyte products are not adsorbed onto the electrode surface, and (d) acetic acid was added to utilize the prepeak current of the DBBQ derived from the acetic acid as a blank in order to avoid variability of the background current on a voltammogram. In addition, a defined prepeak of DBBQ appeared in the real sample analysis using sake when the DBBQ cocktail containing acetic acid was used, but not when citric, malic, or succinic acid was included. Considering this finding, acetic acid was selected as the acidic compound to be added to the DBBQ cocktail.

A linear sweep voltammogram obtained from the DBBQ cocktail is shown in Fig. 2. After a potential sweep was started at +0.4 V vs Ag/AgCl, a reduction prepeak of DBBQ derived from

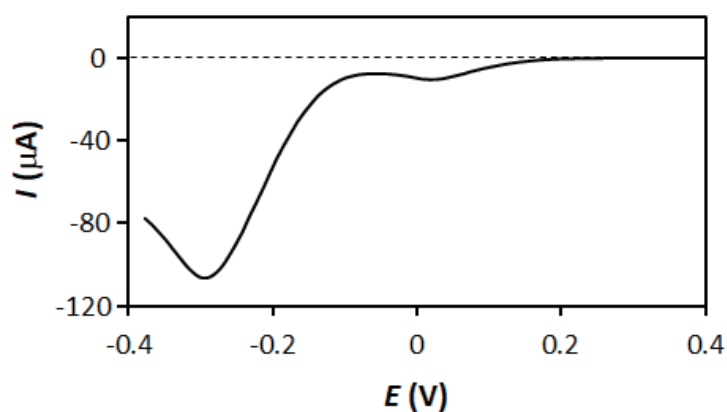


Fig. 2. Linear sweep voltammogram obtained from a DBBQ cocktail [ethanol–water–isopropanol (5:4:1, v/v/v) mixture containing 0.1 M sodium chloride, 15 mM DBBQ, and 1.5 mM acetic acid]. A glassy carbon working electrode, an Ag/AgCl reference electrode, and a coiled platinum wire counter electrode were used in the electrochemical cell. The scan rate was set at 20 mV/s. The zero line of current is shown by the dashed line.

the acetic acid in the DBBQ cocktail appeared at +0.004 V vs Ag/AgCl, and then the main reduction peak of DBBQ appeared at −0.300 V vs Ag/AgCl.

In a similar manner, a linear sweep voltammogram was measured after the addition of succinic acid to the DBBQ cocktail. Because succinic acid is a major organic acid in sake and is available as a reagent with high purity, succinic acid was selected as a standard substance to obtain a calibration curve for determining titratable acidity. As shown in Fig. 3(a), a defined reduction prepeak of DBBQ derived from succinic and acetic acids in the test solution was observed at −0.018 V vs Ag/AgCl. The value of the prepeak current from the zero line [Fig. 3(a), dashed line] at each potential was used for the series of calculations for determining titratable acidity. The prepeak currents of DBBQ were proportional to the concentrations of succinic acid added into the DBBQ cocktail in the range from 1.33 to 33.3 mM with a correlation coefficient (r^2) of 0.999, as shown in Fig. 3(b). The detection limit for succinic acid was estimated as 0.38 mM ($3.3\sigma/a$), where σ and a mean the standard deviation (SD) of prepeak current derived from acetic acid as shown in Fig. 2 and the slope of the calibration curve for succinic acid as shown in Fig. 3(b), respectively. For the determination of titratable acidity using the portable sensor, a calibration curve of succinic acid was used to determine an equivalent concentration of acidic compounds in sake and moromi. Considering the definition of the titratable acidity of sake, the relationship between the titratable acidity (V_A) and the concentration of acidic compounds in sake (C_{SA}), which is obtained from a calibration curve of succinic acid, is expressed as follows:

$$C_{SA(\text{mM})} \times 2 = \frac{100_{(\text{mM})} \times V_A}{10_{(\text{mL})}}, \quad (1)$$

where the factor of 2 was adopted to correct the equivalent concentration, and 100 (mM) and 10 (mL) were used for the concentration of sodium hydroxide titrant and sample volume (sake) in

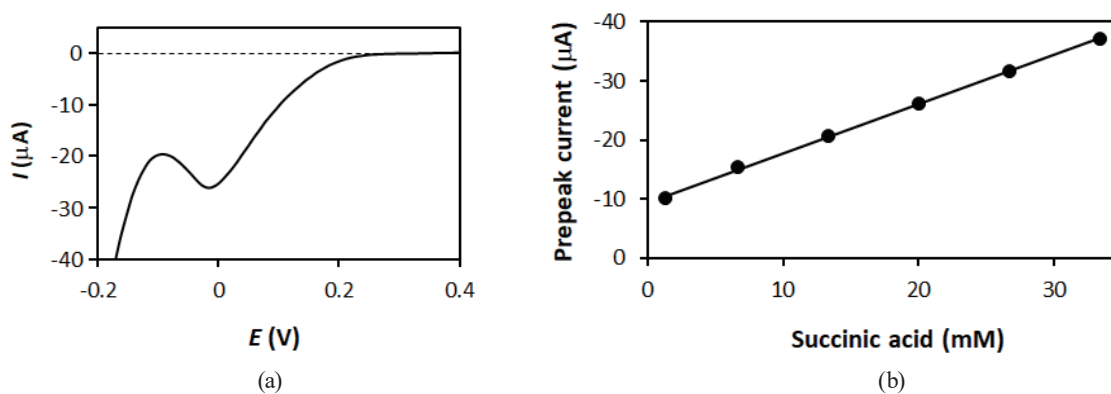


Fig. 3. (a) Linear sweep voltammogram obtained from DBBQ cocktail with 20 mM succinic acid and (b) relationship between prepeak current and concentration of succinic acid mixed with the DBBQ cocktail. (a) The zero line of current is shown by the dashed line. Conditions for voltammetry were the same as in the case of Fig. 2. (b) Error bars showing SDs, which were estimated by repetitive measurements ($n = 3$) using a test solution prepared each time, were within each concentration point.

the official neutralization titration, respectively. Considering the linear range for the calibration curve of succinic acid, the portable sensor can be used to determine the titratable acidity of sake and moromi in the range from 0.266 to 6.66. The linear range of titratable acidity using the reported voltammetric sensor was 0.10–6.8,⁽¹⁵⁾ indicating that the portable sensor has a similar linear range. Thus, it was found that the portable sensor had a sufficient linear range for determining the titratable acidity of sake and moromi.

3.2 Determination of titratable acidity of commercially available sake

A linear sweep voltammogram was measured after the addition of sake to the DBBQ cocktail. A reduction prepeak of the DBBQ derived from the acidic compounds in sake appeared as shown in Fig. 4(a). In the analysis of sake (A), the concentration of acid compounds as a dibasic acid was determined to be 8.38 mM from the prepeak current on the voltammogram and the calibration curve of succinic acid. Thus, the titratable acidity of sake (A) was determined to be 1.7 using Eq. (1).

The titratable acidities of the four types of sake determined using the portable sensor were compared with those determined by the official neutralization titration method. The quantitative results of the titratable acidities of the sake determined using the portable sensor and by the official neutralization titration were essentially the same as shown in Table 1. Moreover, the relative standard deviations (RSDs) of the titratable acidities of the sake determined using the

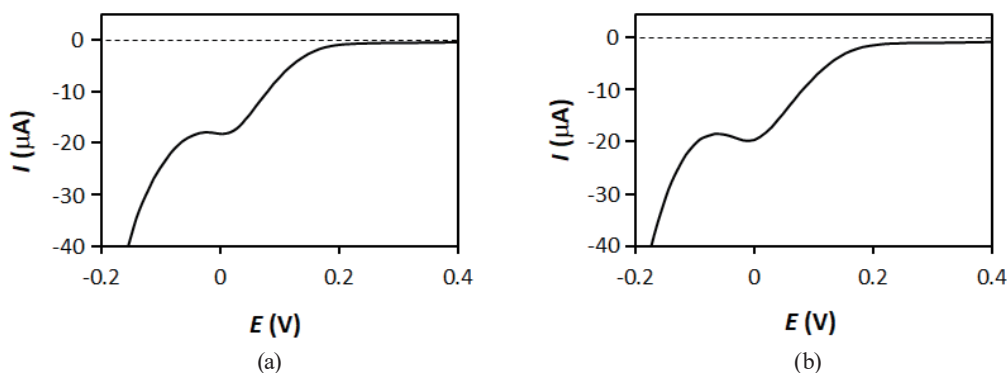


Fig. 4. Linear sweep voltammogram obtained from a DBBQ cocktail with (a) sake (A, Tokyo) and (b) a moromi sample fractionated at 20 days after brewing. The zero line of current is shown by the dashed line. Conditions for voltammetry were the same as in the case of Fig. 2.

Table 1

Titratable acidities of commercially available sakes determined using the sensor and by titration.

Sake	(Prefecture)	Sensor ($n = 3$) ^a		Titration ($n = 3$) ^a	
		Titratable acidity	RSD (%)	Titratable acidity	RSD (%)
A	(Tokyo)	1.7	3.1	1.7	1.6
B	(Niigata)	1.4	2.1	1.3	1.2
C	(Nagano)	1.4	2.0	1.4	1.7
D	(Tokyo)	1.8	1.7	1.8	1.6

^a The test solutions ($n = 3$) were prepared each time, and they were used to perform voltammetry and titration.

portable sensor were less than 3.1% ($n = 3$). Although the RSDs of the titratable acidities determined using the portable sensor were larger than those determined by the official neutralization titration, the portable sensor had sufficient precision to determine titratable acidity for the quality control of sake during the brewing process and for personal use when selecting sake. The RSDs of the titratable acidities of the sakes determined using the reported voltammetric sensor were less than 3.1%,⁽¹⁵⁾ indicating that the portable sensor has a similar precision. By the real sample analyses using commercially available sake, we showed that the portable sensor can be satisfactorily applied to determine titratable acidity with accuracy and precision. As such, the portable sensor would be useful to help select sake for personal use.

3.3 Determination of titratable acidity of moromi samples during brewing

Moromi samples were fractionated from tank #1 at 13, 15, 17, and 20 days after brewing, and then the titratable acidities of these samples were determined using the portable sensor and by the official neutralization titration. Figure 4(b) shows a reduction prepeak of DBBQ derived from the acid compounds in the moromi sample obtained at 20 days after brewing. A defined reduction prepeak of DBBQ was similarly observed in the voltammetric measurement of the same moromi sample. Moreover, titratable acidities of other moromi samples fractionated from tank #2 at 15, 17, 19, 22, and 29 days after brewing were determined using the portable sensor and the official neutralization titration. The titratable acidities of the moromi samples during brewing determined using the portable sensor were compared with those determined by the official neutralization titration. As shown in Table 2, the titratable acidities of the moromi samples determined using the portable sensor and the official neutralization titration were essentially the same. It was found that the portable sensor could be applied to monitor titratable acidity during the brewing process of sake.

To determine the titratable acidity of the moromi samples by the official neutralization titration method, 10 mL of a moromi sample and about 5 min of measurement time were required for one assay. Incidentally, storage of the sodium hydroxide solution while keeping the quality

Table 2
Titratable acidities of moromi samples determined using the sensor and by titration.

Brewing day	Sensor ($n = 3$) ^a		Titration ($n = 3$) ^a	
	Titratable acidity	RSD (%)	Titratable acidity	RSD (%)
(Tank #1)				
13	2.2	4.2	2.3	0.3
15	2.4	1.8	2.3	0.3
17	2.1	1.3	2.3	0.3
20	2.3	3.3	2.3	1.2
(Tank #2)				
15	1.9	4.8	1.9	0.3
17	1.7	3.3	1.9	0.5
19	1.8	4.0	1.9	0.3
22	1.7	2.5	1.7	0.1
29	2.0	2.7	1.8	0.6

^a The test solutions ($n = 3$) were prepared each time, and they were used to perform voltammetry and titration.

constant is difficult, hindering accurate neutralization titration. In the case of the portable sensor, 0.3 mL of a moromi sample and about 1 min of measurement time were required for one assay. Also, the DBBQ cocktail was stable for 3 months when it was stored in the dark at room temperature. Thus, the determination of the titratable acidity of a moromi sample using the portable sensor has advantages from the viewpoints of saving on sample amount and reducing measurement time compared with the official neutralization titration method. We have shown that the portable sensor is useful as an on-site means of analysis to control the brewing process of sake.

4. Conclusions

In this study, a portable sensor based on the measurement of the reduction prepeak of DBBQ in the presence of acidic compounds was developed for determining the titratable acidity of sake and moromi. Real sample analyses using the portable sensor, to determine the titratable acidity of commercially available sakes and moromi samples during brewing, were performed to demonstrate the practical utility of the portable sensor. The titratable acidities of the real samples determined using the portable sensor were essentially the same as those determined by the official neutralization titration method of the National Tax Agency Japan. Furthermore, it was shown that the portable sensor had sufficient accuracy and precision as a quantitative sensor for determining the titratable acidity of sake and moromi. The portable sensor was also shown to be applicable for the quality control of sake during the brewing process and for personal use to help select sake using the measured titratable acidity as a criterion.

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