Electrochemical antioxidant capacity measurement: a downsized system and its application to agricultural crops

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Abstract
An on-site electrochemical antioxidant capacity measurement system was developed using a screen print electrode (SPE) and circuit tester. The antioxidant capacities of eight antioxidants were evaluated with the handheld electrochemical antioxidant capacity measurement system to compare with those measured with spectroscopic methods, namely, oxygen radical absorbance capacity (ORAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays, as well as the reported electrochemical method with three conventional electrodes (a glassy carbon electrode, Ag/AgCl electrode and platinum wire electrode) and a potentiostat. Additionally, the potential shifts were proportional to the logarithm of the antioxidant concentration, which obeyed the Nernstian equation. Moreover, the antioxidant capacities of extracts from vegetables (green pepper, ginger and eggplant) were measured with a handheld electrochemical system. Each measurement was finished in only ca. 3 min. The electrochemically obtained antioxidant data were comparable to those from DPPH free-radical scavenging assays and superoxide anion scavenging activity (SOSA) assays, as well as the total phenolic compound content.

Keywords Antioxidant capacity · Electrochemical measurement · Screen print electrode · Handheld · Agricultural crops

Introduction
Many people are interested in the intake of food, beverages and supplements containing antioxidants to avoid the risk of diseases generated from oxidative stress [1–5]. Various types of antioxidant capacity measurements, such as oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays and Folin-Ciocalteu (F–C) methods, have been developed. [6–8] Although many researchers have used such spectrophotometric antioxidant capacity measurements, most spectrophotometric methods have potential issues, such as the need for high skill levels to obtain values with high reproducibility and precision, long measurement times (ca. 2 h), incidence of serious errors due to complex and coloured matrices and expensive equipment. Therefore, most producers of crops, food and beverages cannot measure antioxidant capacities easily, such as handheld sugar content meters and salt meters, although several automated DPPH free-radical scavenging assays and new rapid antioxidant activity measurement methods using near- and mid-infrared spectroscopy have been developed. [9, 10].

Recently, we developed an electrochemical antioxidant capacity evaluation method using polyoxometalates (POMs) as probes, and it has high prospects to mitigate issues related to spectrophotometric antioxidant capacity measurements [11]. POMs are anionic inorganic clusters that consist of tungsten and molybdenum in the framework, phosphorus...
and sulfur in the center and oxygen [12–15]. POMs exhibit excellent electrochemical properties, such as multielectron transfer and proton and lithium-ion coupling, depending on their reduced levels, which lead to their application in catalysis, materials and sensors [16, 17]. Electrochemical antioxidant capacity measurements were achieved using the shift in the open-circuit potential or the limiting potentials generated from the reaction of POMs with antioxidants, as follows: [11]

\[ \text{POM(Ox)} + \text{Antioxidant} \rightarrow \text{POM(Red)}, \]

\[ E = E^0 + \frac{0.059}{2} \log \left( \frac{[\text{POM(Ox)}]}{[\text{POM(Red)}]} \right). \]

However, a conventional three-electrode system comprising a glassy carbon electrode as the working electrode, an Ag/AgCl electrode as a reference electrode and a platinum wire electrode as a counter electrode was used with a potentiostat. The measurement required several minutes, indicating that the reported method may not be appropriate for on-site measurements. Currently, various types of compact screen print electrodes (SPEs), which consist of carbon, precious metals and conducting polymers, are commercially available and have led to applications in various sensors [18–22]. In addition, if only the potential is measured, a circuit tester is sufficient, removing the need for a potentiostat.

In this study, a downsized electrochemical antioxidant capacity measurement system was developed using a circuit tester and SPEs to enable on-site measurements anywhere, such as on farms and in food factories. The measurement conditions were also optimized to achieve rapid data acquisition with high reproducibility. The obtained data were compared with those from a previously established electrochemical method as well as ORAC and DPPH methods. In addition, the antioxidant capacities of the extracts from vegetables, including green pepper, ginger and eggplant, of which the yield harvested in Kochi Prefecture is the highest in Japan, were measured with the developed handheld electrochemical antioxidant capacity measurement system and compared with those from DPPH free-radical scavenging assays and superoxide anion scavenging activity (SOSA) assays and the total phenolic compound content.

**Experimental**

Based on the optimized conditions for a solution containing POMs reported previously [11], POM solutions were prepared as follows: Stock solutions of Mo(VI), W(VI) and P(V) were prepared by dissolving Na2MoO4·2H2O, Na2WO4·2H2O, and NaH2PO4·2H2O in water. The V(V) stock solution was prepared by mixing V2O5 in NaOH solution in a Teflon beaker. For the [PMo12O40]3−(PMo12) solution, a 100-mL solution of 10 mM Mo(VI), 1 mM P(V), 0.1 M HCl, and 50% (v/v) EtOH was allowed to stand for 1 day at room temperature before measurement. For the [PVW11O40]4−(PVW11) solution, a 100-mL solution of 10 mM W(VI), 1 mM P(V), 1 mM V(V), 0.1 M HCl, and 50% (v/v) EtOH was heated in a sealed bottle at 70 °C for 1 day. For the [SV2W10O40]4−(SV2W10) solution, a 100-mL solution of 10 mM W(VI), 1 mM V(V), 0.1 M H2SO4, and 50% (v/v) EtOH was heated at 70 °C for 1 day. All solutions were cooled to room temperature prior to use.

Analytical-grade ascorbic acid, ellagic acid, gallic acid, trans-ferulic acid, morin hydrate, catechin hydrate, quercetin, and sesamol standards were purchased from Fujifilm-Wako and Tokyo Chemical Industries and used as received without any purification. Stock solutions (0.01 M) of antioxidants were prepared by dissolving ascorbic acid, gallic acid and catechin hydrate in water and the others in ethanol, stored in a refrigerator and used only for a week. For ascorbic acid, the aqueous stock solution was freshly prepared every day prior to a measurement. Extracted solutions of vegetables were prepared as follows: Vegetables were washed with water and cut into a ca. 1 cm3 cubic shape. Then, 20 g of cubed vegetables was put in a microwave-safe container, followed by the addition of 200 mL of Milli-Q water and heating under microwave irradiation at 500 W for 3 min. After mixing the sample for 10 s, it was filtered with cotton to separate the residue. The filtrate was filtered again with a 0.45 μm pore size membrane filter (Starlab Co. Ltd.).

SPEs [Metrohm DropSens: model DRP-110(C(WE), Ag(RE), C(CE)), DRP-150(C(WE), Ag(RE), Pt(CE)) and DRP-AUTR10(Au(WE), Ag(RE), C(CE)), where WE is a working electrode, CE a counter electrode and RE a reference electrode, respectively, were used for electrochemical measurements and were connected with a DRP-DSC interface box to communicate with a potentiostat (HOKUTO DENKO Co., model HA-501) or a circuit tester (SANWA Electric Instrument Co., Ltd., model PC700). Only DRP-AUTR10 was attached on the backside of DRP-110 or DRP-150 due to reinforcement of the thin electrode. The ring-shaped, silicon-walled tube (10 mmφ) was cut to ca. 1 cm and adhered around the SPEs with a strong glue (Aron Alpha Extra2000, TOAGOSEI Co., Japan) to keep the solution from spilling out when mixing, as shown in Fig. 1.

The DPPH antioxidant assay kit and superoxide dismutase (SOD) assay kit-WST purchased from DOJINDO laboratories were used for DPPH free-radical scavenging assays and SOSA assays, respectively. Both assays were measured in accordance with the procedure manuals. The total phenolic compound content was measured with the modified F–C method. Fifty microliters of a sample solution and a 0–100 g/mL gallic acid solution as the standard sample...
were added to a 96-well microplate followed by the addition of 50 μL of F–C phenol reagent, which was diluted in 1/20 of the original concentration, and 100 μL of 0.175 M NaOH aqueous solution. The solutions in wells were mixed using a microplate shaker at 30 °C for 1 min and then incubated at room temperature for 3 min. The absorbance at 725 nm was recorded by a microplate reader (iMark™ Microplate Reader, Bio-Rad Co. Ltd.). The total phenolic compound content was expressed as the corresponding gallic acid amount (mg GAE/g f.w.).

Results and discussion

When 30 µL of a PMo₁₂ solution was dropped onto the three types of SPEs, the colour of the solution changed from yellow to blue in a few minutes. This colour change indicated the reduction of PMo₁₂. Because silver, which was used as the reference electrode of the three SPEs, could react with PMo₁₂, and the potentials were measured at two points using a potentiostat or circuit tester to simplify the measurement procedure, the silver electrode (RE) was sealed with strong glue. Moreover, after 30 µL of PMo₁₂ solution was dropped onto the three SPEs with the sealed reference electrode, the change in the solution colour was similarly changed in the case of DRP-100 and DRP-150, while no colour change occurred for PMo₁₂ on DRP-AUTR10. Silver or the other materials used under WE and CE in DRP-100 and DRP-150 would lead to a reduction in PMo₁₂ [23]. In this study, only DRP-AUTR10 was used for further experiments.

The change in the limiting potentials was investigated by a potentiostat with connected reference and counter plugs, and measured over time after the addition of PMo₁₂, PVW₁₁ and SV₂W₁₀ solutions (30 μL) to the DRP-AUTR10 SPE. For all POMs, a drastic change in potential was observed 1 min after the addition of POM solution. The differences in the potentials from those observed at 1 min after addition were recorded every minute. Triplicate experiments were conducted for each POM solution (Fig. 2). For the SV₂W₁₀ solution, the potential drifted only ca. 10 mV for 5 min, so the SV₂W₁₀ solution was chosen for subsequent experiments.

The limiting potentials of eight antioxidants were investigated in SV₂W₁₀ solution (Fig. 3). One hundred microliters of SV₂W₁₀ solution was dropped onto the DRP-AUTR10 SPE, and the potential was measured every 10 s by a circuit
tester with a common terminal and a measuring terminal connected to CE and WE, respectively. The limiting potential almost plateaued \((E_0)\) 80 s after the addition of \(SV_2W_{10}\) solution. Two microliters of antioxidant solution was added to the \(SV_2W_{10}\) solution on the DRP-AUTR10 electrode ca. 120 s after starting the measurement, and then the potential was measured every 10 s. The potential achieved a local minimal value \((E_1)\) in 30–60 s, implying that antioxidant capacity evaluation could be completed in only 2–3 min for each sample. The antioxidant capacity was evaluated as \(\Delta E = |E_0 - E_1|\). All of the obtained \(\Delta E\) values, which were measured at least three times, were checked by the Q-test, and only values without any statistical anomalies are listed in Table 1. These data were compared with those obtained by the electrochemical method with a traditional three-electrode system reported previously and by the ORAC method (Fig. 4) [11]. Similar to a previous report, the obtained potentials were linearly related to the ORAC values with reasonably high correlation coefficients except for that of ascorbic acid. The mechanism for the antioxidant action of ascorbic acid was different from that of the other tested antioxidants, which could lead to an underestimation of the antioxidant capacity of ascorbic acid by the ORAC and DPPH methods. In addition, the potentials obtained in this study were highly correlated to those from the previously reported electrochemical method with a three-electrode system, yielding a high correlation coefficient, although the measurable potential range became narrower due to the different bias currents and circuits of the circuit tester with a two-electrode system and SPE and the potentiostat with a three-electrode system and three types of solid electrodes: glassy carbon, platinum wire and Ag/AgCl electrode. Moreover, potentials were measured for specific concentrations of four antioxidants. The potentials were proportional to the logarithm of the antioxidant concentration (Fig. 1S); however, two different slopes were obtained for catechin and sesamol due to the different reduction ratios of \(SV_2W_{10}\) at low antioxidant concentrations. The change in the potentials obeyed the following chemical reaction and Nernst equation:

\[
E = E^0 + \frac{0.059}{2} \log \frac{[SV_2W_{10}(Ox)]}{[SV_2W_{10}(Red)]}.
\]

Therefore, this electrochemical method can evaluate both antioxidant power and the concentration. The developed handheld electrochemical antioxidant evaluation system exhibited sufficient performance to investigate the antioxidant capacity of agricultural crops on site.

The antioxidant capacities of extracted solutions from vegetables (eggplant, ginger and green pepper) harvested in Kochi Prefecture, Japan, were evaluated by the developed system. The limiting potential was almost a plateau \((E_0)\) 80–120 s \((E_0)\) after the addition of the \(SV_2W_{10}\) solution to

### Table 1 Differences between the initial potentials and limiting potentials of \(SV_2W_{10}\) in the presence of 0.1 mM antioxidants in the Au electrode

| Antioxidants  | Circuit tester (two electrodes) | Potentiostat (three electrodes) | ORAC  |
|---------------|---------------------------------|---------------------------------|-------|
|               | \(\Delta E/mV\) | RSD\(^a\) | \(\Delta E/mV\) | RSD\(^b\) | \(\mu mol\) TE/g |
| Ascorbic acid | 71.0 ± 1.0 | 1.4 | 214 | 0.13 | 3011 |
| Catechin      | 44.3 ± 4.3 | 9.8 | 47.0 | 0.57 | 10,676 |
| Quercetin     | 37.3 ± 2.8 | 7.4 | 90.0 | 0.34 | 13,896 |
| Morin         | 37.2 ± 3.4 | 9.0 | 96.0 | 0.13 | 12,583 |
| Gallic acid   | 30.0 ± 1.9 | 6.4 | 56.0 | 0.24 | 10,419 |
| Sesamol       | 13.7 ± 0.9 | 6.2 | 61.0 | 0.44 | 9,049  |
| Ellagic acid  | 12.3 ± 2.8 | 23 | 21.0 | 0.60 | 9,049  |
| Trans-ferulic acid | 5.5 ± 1.5 | 28 | 9.0 | 0.24 | 3,419 |

\(^a\)Relative standard deviation (\%)  
\(^b\)See reference [11]

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**Fig. 4** Comparison of the potential shift values of antioxidants obtained by the handheld electrochemical antioxidant capacity evaluation system with the DRP-AUTR10 electrode and \(SV_2W_{10}\) solution with the corresponding ORAC values (A) and those (B) obtained by the reported electrochemical antioxidant capacity evaluation system.
the DRP-AUTR10 SPE. After the addition of 2 μL of vegetable extract solution into $SV_{2}W_{10}$ solution, the potential suddenly decreased to a minimum ($E_{1}$) in approximately 60 s (Figs. 2S–4S). Then, the potential gradually increased with time. The antioxidant capacity was evaluated from the differences in the potentials ($\Delta E = |E_{0} - E_{1}|$) between the initial potential and the minimum potential, similar to the case of antioxidants. In addition, the antioxidant capacities were also investigated through the DPPH radical scavenging activity and SOSA assays and the total phenolic compound contents for the same extracted samples (Tables 1S–3S). Moreover, the relationship between $\Delta E$ and the DPPH radical scavenging activity, SOSA and total phenolic compound content (Fig. 5) was examined, and acceptable correlation coefficients were observed ($0.43 < r < 0.76$). Antioxidant capacities and total phenolic compound contents for the extracted solutions from each vegetable species were proportional to the potentials. However, when comparing the different vegetables, the antioxidant capacities estimated from the potentials did not reflect those from the DPPH radical scavenging activity, SOSA and total phenolic compound content. The differences and similarities between the antioxidant capacities obtained with different evaluation methods have been found to depend on the sensitivity of a method for various types of compounds in many cases [6, 24–30]. The cause of the relatively low correlation between the electrochemically obtained antioxidant capacities and spectrophotometrically obtained antioxidant capacities when different types of vegetables were compared has not been elucidated until now, although the detailed mechanism should be investigated in future research.

**Conclusions**

A downsized (handheld) electrochemical antioxidant capacity evaluation system was developed using a circuit tester and SPE (DRP-AUTR10, Metrohm Co. Ltd.) with an Ag reference electrode sealed with a ring-shaped, silicon-walled tube around the electrodes as well as a small amount of water/ethanol $[SV_{2}W_{10}O_{40}]^{4-}$ solution as a potential probe. This system achieved a short measurement time (<3 min per sample), portability and low cost, which overcame the issues of spectrophotometric measurements, such as the ORAC and DPPH methods and a previously published electrochemical measurement method. The antioxidant capacities of the extracts from green pepper, ginger and eggplant were also measured with the handheld electrochemical antioxidant capacity system to compare with those from the DPPH free radical scavenging assay, SOSA assay and total phenolic compound content. As long as the same type of vegetable was compared, the antioxidant capacities were sufficiently appropriate to be evaluated by the developed system, indicating that the system exhibits high performance for on-site antioxidant capacity measurements, similar to a sugar content meter or salt meter.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.2116/analsci.21P217.

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