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Calcium K-edge X-ray Absorption Spectroscopy and Electrochemical Reaction of Green Vegetables

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Green vegetables contain relatively high amounts of calcium. To analyze the chemical species of calcium in spinach (*Spinacia oleracea*), Malabar spinach (*Basella alba*), and Komatsuna (*Brassica rapa* Var. *perviridis*), the calcium K-edge X-ray absorption near edge structures (XANES) were measured using the dried powder samples of these vegetables. The XANES of calcium carbonate, calcium lactate, ascorbic acid calcium, and calcium oxalate were used as reference spectra for comparison. The XANES of spinach and Malabar spinach were consistent with the shapes of calcium oxalate. Komatsuna was similar to the shapes of ascorbic acid calcium and calcium lactate. The electrochemical behavior of these vegetables was also examined. The oxidation peak current, which can be attributed to calcium extraction, was the largest in Komatsuna among them. The calcium K-edge XANES of Malabar spinach treated with different potentials was investigated to analyze the chemical state change of calcium species induced by electrochemical reactions, but it did not show electrochemical potential dependency.

1. Introduction

Along with its presence in organisms and plants, calcium is a naturally abundant resource and a popular element utilized in industries such as cement. Recently, the electrochemistry of calcium ions has gained much attention considering the potential use of calcium metal as a negative electrode in future rechargeable batteries. [1, 2] However, material development for positive electrodes using inorganic materials has stagnated, and new concepts for the material design are needed to realize the calcium rechargeable battery.

Green vegetables categorized as *Spinacia* or *Brassicaceae* generally contain large amounts of calcium compared to other vegetables. [3] Among green vegetables, spinach (*Spinacia oleracea*), Malabar spinach (*Basella alba*), and Komatsuna (*Brassica rapa* Var. *perviridis*) contain relatively large amounts of calcium and iron, which might contribute to electrochemical reactions as a redox center. Therefore, it would be meaningful to analyze the relationship between chemical structure and electrochemical behavior. Many studies indicate that spinach contains calcium oxalate; [4] however, to our knowledge, the chemical species of calcium in Malabar spinach and Komatsuna have not been clarified. Although there have been several reports of calcium K-edge X-ray absorption spectroscopy (XAS) in plants, [5, 6] studies comparing several green vegetables are not available. In this study, the electronic structure of spinach, Malabar spinach, and Komatsuna was evaluated by calcium K-edge XAS, and their chemical species were elucidated using a fingerprinting technique. Their electrochemical behavior as electrode materials was also analyzed.

2. Experimental

Purchased spinach, and self-grown Malabar spinach and Komatsuna were used in the study. The leaves were vacuum dried for 24 h at 75 °C, and ground in a mortar to prepare powdered samples. Further, calcium carbonate, calcium lactate pentahydrate, calcium L-ascorbate dihydrate, calcium oxalate monohydrate (all purchased from Fujifilm Wako Pure Chemical Industries, Ltd.) were used as references. Hereafter, these are referred to as calcium carbonate, calcium lactate, ascorbic acid calcium, calcium oxalate, respectively.

Calcium K-edge XAS was performed at BL-13 in the fluorescence mode using a silicon drift detector with the sample at an 85-degree angle to the incoming beam and the fluorescence detector. The incident energy was tuned with a two crystal Ge (111) monochromator. Powder samples were bound to a stainless-steel sample holder via a carbon double stick tape.

Electrodes for electrochemical measurement were prepared as the mixture of vegetable powder: acetylene black: PTFE = 75: 15: 10 (*wt*%) and sandwiching the sheet with a titanium mesh. Using 2.5 M of $Ca(NO_3)_2$ aqueous solution as an electrolyte, a three-electrode cell was assembled. The Ag/AgCl double junction electrode and activated carbon were used as reference and counter electrodes, respectively. Electrochemical measurements were performed using a Hokuto Denko HZ-7000. Cyclic voltammetry was performed at a sweep rate of 10 mV/s and in the potential ranges of -0.8 to 0.9 V.

3. **Results and Discussion**

The X-ray absorption near edge structure (XANES) at the calcium K-edge of the reference materials was compared with the previously reported XANES. Figure 1 shows the calcium K-edge XANES of calcium carbonate, calcium lactate, ascorbic acid calcium, and calcium oxalate. The XANES of calcium carbonate has large absorption main peaks around 4038 and 4049 eV, indicated by B and C in Fig. 1. A pre-edge peak (denoted as A) is seen around 4033.5 eV on the lower energy side of the main peak. On the higher energy side, the broad peaks D, E, and F around 4069, 4083, and 4093 eV, respectively, are confirmed. Hayakawa *et al.* have reported that calcium carbonate has calcite, aragonite, vaterite polymorphs, each differing in the calcium K-edge XANES. [7] The shape of XANES measured in this study is consistent with that reported for calcite. For the XANES of calcium lactate, there is a major peak H at 4039 eV and a broad structure at 4046 eV. A pre-edge peak G can be further identified at 4028 eV. On the higher energy side, there are broad peaks at 4067 and 4083 eV. These shapes are similar to the XANES of calcium lactate reported by Zhang *et al.* [8] This structure is also similar to the XANES of calcium pectate, which is generally present in cell walls, reported by Punshon et al. [6] The XANES of ascorbic acid calcium has a sharp single peak L at 4039 eV and broad peaks at 4052, 4069, and 4081 eV. To the best of our knowledge, the XANES of ascorbic acid calcium has not been reported. Although the main peak of 4039 eV and the shape at higher energy of the main peak are similar to that of calcium lactate, the structure of calcium oxalate differs in the peak R, S, and T located at the higher energies. Abe *et al.* reported the calcium K-edge XANES of calcium oxalate, [5] which shows a good agreement in the arrangement of these peaks. Thus, the calcium K-edge XANES of calcium compounds has respective characteristic peaks, and this information can be utilized for fingerprinting analysis.

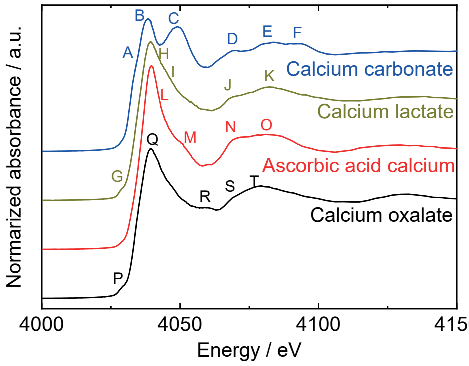

Fig. 1 Calcium K-edge XANES of calcium carbonate, calcium lactate, ascorbic acid calcium, and calcium oxalate.

Figure 2(a) shows the calcium K-edge XANES of the measured vegetables. The XANES of spinach and Malabar spinach is similar as indicated by the main peak at 4039 eV, the peak at 4060 eV and the broad peaks at 4069 and 4078 eV. In addition, the XANES of Komatsuna shows the main peak at 4039 eV and the broad peaks at 4072 and 4080 eV. These XANES are compared with the reference samples as shown in Fig. 2(b) and (c). According to fingerprinting analysis, the XANES of spinach and Malabar spinach are consistent with the XANES of calcium oxalate, indicating calcium oxalate as the calcium species in both vegetables. This finding is similar to the conclusion by Abe *et al.*, for the

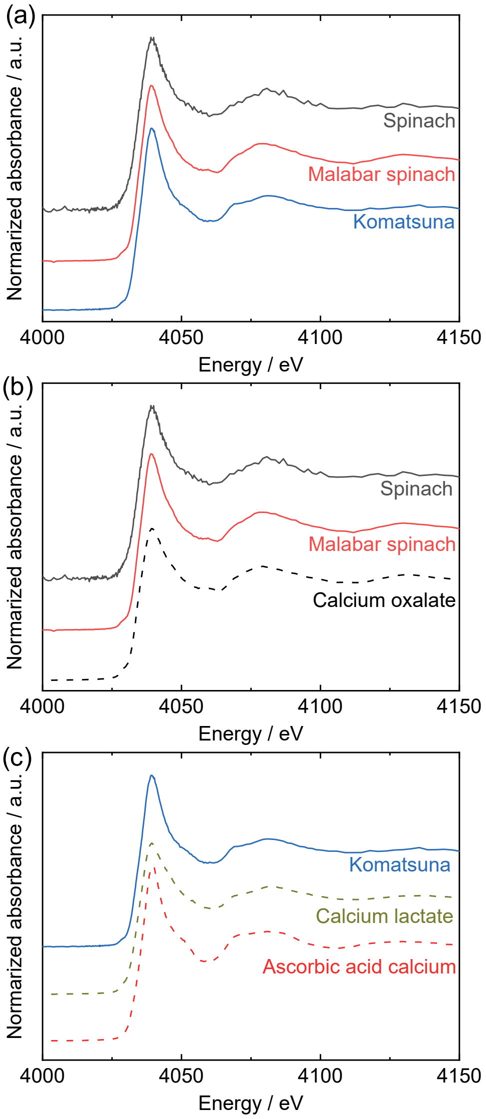

Fig. 2 (a) Calcium K-edge XANES of spinach, Malabar spinach, and Komatsuna. (b), (c) Comparison of these vegetables with the reference samples.

XANES of raw spinach. [5] Although this study does not discuss the chemical change during drying process, the similar results imply no change of calcium organic acid salts. SEM analysis of crystallized spinach and Malabar spinach also showed crystals presumed as calcium oxalate, [9] which agrees with our results. On the contrary, the XANES of Komatsuna is similar to that of calcium lactate or ascorbic acid calcium as shown in Fig. 2(c). The peak observed at 4052 eV is similar to the peak M of ascorbic acid calcium. Comparing the structures on the higher energy side, the shape around 4075 eV is close to the sharp structure observed for calcium lactate. Komatsuna has been reported to contain a soluble calcium, [9] which is consistent with our observation implying that Komatsuna contains water-soluble calcium ascorbate. However, we cannot exclude the possibility of calcium pectate because in general, calcium in plants exists as calcium pectate. The XANES of calcium pectate is similar to that of calcium lactate. [6]

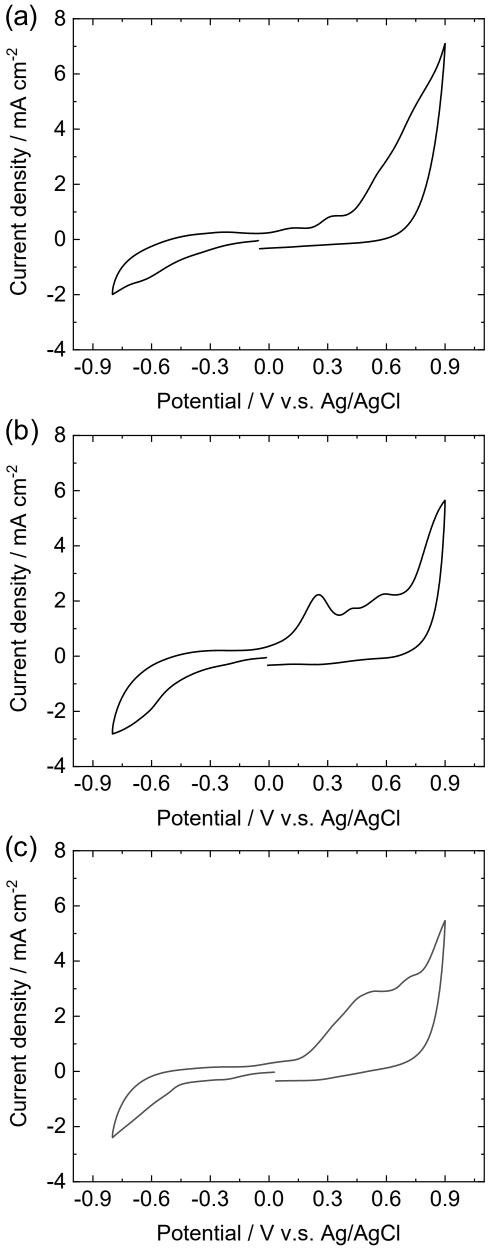

Fig. 3 Cyclic voltammograms of (a) spinach, (b) Malabar spinach, and (c) Komatsuna. The scan rate was 10 mV s^{-1}.

We compared the electrochemical behavior of the three vegetables based on the cyclic voltammograms of spinach, Malabar spinach, and Komatsuna shown in Fig. 3 (a), (b), and (c) respectively. All samples are first swept to the cathode and then to the anode. No distinct reduction peaks were observed, whereas the anodic sweep confirmed the distinct peaks. The anodic peaks were observed around 0.3 V for spinach and Malabar spinach, and around 0.5 V for Komatsuna. A higher anodic current was observed, particularly in Komatsuna which has different chemical species of calcium, and the peak position was shifted towards the positive direction. Among the three vegetables, the calcium content is in the order Komatsuna, Malabar spinach, and spinach, which is consistent with the order of the peak currents. When this anodic current is assumed to be derived from the electrochemical deinsertion of calcium ions, approximately 30 to 50% of the calcium content is estimated to be extracted in this reaction.

To discuss whether the redox species was iron-ion, we examined the Fe K-edge and Fe L-edge XANES but the signal was too weak to be analyzed. The further analysis about the redox species is needed. In this study, we measured the calcium K-edge XANES of Malabar spinach electrochemically treated at different potentials to discuss the chemical change of calcium. Figure 4 shows the calcium K-edge XANES of dried Malabar spinach electrodes after holding at different potentials compared to the powder sample. No potential dependence was

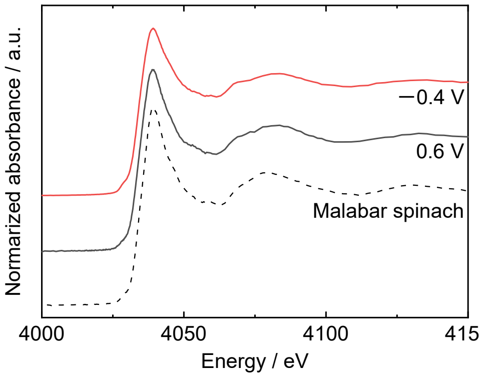

Fig. 4 Calcium K-edge XANES of Malabar spinach after electrochemical measurement.

detected in the spectral shapes of the electrode samples, and the characteristic structures detected in Malabar spinach powder at 4060 and 4069 eV were not observed. Although the reason was not clarified, we suspect that the washed electrode still contained remained electrolyte salts after the electrochemical insertion-deinsertion, which requires further investigation.

4. Conclusions

The calcium K-edge XANES of calcium carbonate, calcium lactate, and calcium oxalate were consistent with the reported XANES. The XANES of ascorbic acid calcium was reported for the first time in this study. The XANES of dried powder samples of spinach, Malabar spinach, and Komatsuna were compared with the reference materials. While the XANES of spinach and Malabar spinach were consistent with the shapes of calcium oxalate, the XANES of Komatsuna was similar to the shapes of ascorbic acid calcium and calcium lactate. Thus, in spinach and Malabar spinach, calcium exists as calcium oxalate, whereas that in Komatsuna exists as ascorbic acid calcium or calcium lactate. The electrochemical behavior of these vegetables indicated that Komatsuna had the highest anodic current. The calcium K-edge XANES of Malabar spinach did not show electrochemical potential dependency.

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