博士論文要約（Summary）

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専攻 Course：農水圏資源環境科学専攻 Resource and Environmental Science of Agriculture Forestry and Fisheries

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タイトル	機能水による抗酸化特性に関する基礎ならびに応用研究
Title	A Fundamental and Applied Study on the Antioxidant Property of Functional Water

キーワード Key word（Antioxidant activity）（Alkaline electrolyzed water）（Tourmaline water）（Functional water-modified juice）（Silver ionized water）

Introduction and Purpose

Reactive oxygen radicals induce oxidative stress and increase the risk of lifestyle diseases (hypertension, diabetes, coronary heart disease, cancer, etc.). Antioxidants, which neutralize or destroy reactive oxygen radicals before they damage the DNA in cells, have become an essential part of life. Recently, a number of scholars have focused on exploring methods that can improve the antioxidant activity. Moreover, some functional waters exhibit superoxide dismutase (SOD)-like activity. In the present research, a series of studies were conducted to investigate the antioxidant activity of functional water. Alkaline electrolytic water (AlEW) and its composition were verified from physicochemical measurements, and it was found that the pH and characteristic changes of the adjusted water produced some differences in the glass electrode pH meter (GpH meter) and colorimetric method. Therefore, the cause of the improved antioxidant activity of AlEW was evaluated by the WST-kit method depending on the generation conditions and constituent factors, and its antioxidant activity was compared with that of tourmaline treated water (TMW). The feasibility of functional water-improved antioxidant property was verified in food industry. By preparing the concentrated reconstituted juices, the antioxidant activity, processing and storage properties, and sensory evaluation of functional water-modified juices were investigated. Moreover, a novel functional silver ionized water (SIW) generated by microcurrent treatment (5 mA) was developed and its antioxidant properties were evaluated.

The purposes of this study were to investigate the antioxidant activity of functional water, to evaluate the changes in the characteristics of AlEW, to analyze the causes of the improved antioxidant activity of AlEW, to confirm the application of functional water in the food industry, and to provide a basis for the development and utilization of functional water.

Materials and method

Preparation of sample solutions

Pure water: Generated by a pure water generator. pH-adjusted water : 180 mL of pure water was added to a flask containing 20 mL of 0.1 mol/L sodium hydroxide aqueous solution to produce 200 mL of 0.01 mol/L sodium hydroxide aqueous solution (pH 12). Thereafter, 4/5-fold dilution (14 times) was repeated, and to make the pH value range from 10.0 to 11.6 (hereinafter referred to as “ionpH”). Sodium chloride aqueous solution : Salt solution with a salt concentration of 0 to 0.4% was produced using sodium chloride solution. Bubble water : Generated by circulating 0.4 L/min of hydrogen, air, and nitrogen into pure water using a pump swirling shear type bubble generator for 30 minutes. Alkaline Electrolyzed water (AlEW): AlEW was generated at a current of 6-18 A at a voltage of 10 V with an electrolyzed water generator. Tourmaline water (TMW): 770 g of Brazilian tourmaline stone was kept in water bath (T-25, Thomas Kagaku Co.Ltd., Tokyo, Japan) at 25, 50, 75, 100 ℃ for 30 min, and 7.7 L of pure water was circulated for 30 minutes at 2.2~2.4L/min by magnet pump (PMD-1521BNP1, Sanso Electric Co., Ltd., Hyogo, Japan). Silver ionized water (SIW), AlEW-SIW and TMW-SIW were generated from the electrolytes of tap water, AlEW and TMW, respectively.

Difference of pH Value of Alkaline Electrolyzed Water by Glass Electrode pH Meter and Colorimetry

Forty milliliters of pH-adjusted water was added to 50 mL centrifuge tubes, and the sample temperature was adjusted to 20, 30, and 40°C. Then, after staining with Alizarin Yellow R solution, the absorbance was measured at 497 nm. Substitute the absorbance value into the conversion formula obtained in determination of maximum absorption wavelength to calculate the colorimetric conversion pH. A standard curve was drawn at each temperature, and the colorimetric pH value was compared with the measured pH value of the glass electrode pH meter (TPX-999, Toko Chemical Laboratory Co., Ltd., Tokyo, Japan) to calculate the temperature error caused by the glass electrode pH meter. Each treatment was repeated three times.

Measurement of SOD activity of each sample solution

The SOD Assay Kit-WST (Water-Soluble Tetrazolium) was used to measure the antioxidant ability. SOD Assay Kit - WST is a method for measuring superoxide radicals produced by xanthine oxidase (XO) using tetrazolium salts. When the superoxide radical causes an oxidation reaction, the reduction reaction of WST-1 to WST-1 formazan occurs and the color turns yellow. In this research, ascorbic acid (AsA) was dissolved in the sample solution at a concentration of 2 mmol/L. The antioxidant activity of each sample solution was measured and the factors affecting the improvement effect of each sample solution on the antioxidant activity of AsA were analyzed, such as salt solution concentration, pH, hydrogen bubble, electrolysis intensity, and tourmaline stone treatment temperature. The specific usage method of SOD Assay Kit-WST is as follows. First, 20 µl sample solution (sample, blank 2) or pure water (blank 1, blank 3) filled individual holes in the 96-well plate. Second, added 200 µl of WST working solution (Dilute 1 ml of WST solution with 19 ml of Buffer solution) to each well and mixed well with pipetting or plate mixer. Next, added 20 µl of Dilution buffer to the wells of blank 2 and blank 3. And then, added 20 µl of Enzyme working solution (Dilute 15 µl of Enzyme solution with 2.5 ml of Dilution buffer) to the well containing the sample solution and the well of blank 1. Finally, incubated at 37 °C for 20 minutes, and measured the absorbance at 450 nm with a microplate reader (MPR-A100, AS ONE Co., Ltd., Osaka, Japan). The SOD Activity (Inhibition Rate) % is calculated by the following formula.

$$SOD\ Activity\ (Inhibition\ Rate)\% = \frac{\left[(A_{blank1} - A_{blank3}) - \left(A_{sample} - A_{blank2}\right)\right]}{(A_{blank1} - A_{blank3})} \times 100$$

A_{blank1} is the absorbance of blank1.

Application of functional water in concentrated fruit juice

The fresh fruits were washed, the orange peel was removed, the oranges and apples were cut into pieces, and the juice was squeezed using a juicer. Filtered (100-mesh sieve) Tropicana and freshly squeezed juice (hereafter collectively referred to as "juice") were used to remove impurities such as pulp. The physical properties of the original juice were first measured, and then the juice was concentrated. In this experiment, the concentrated juice was prepared using the freeze concentration method. In brief, the juice was frozen in a refrigerator at −20 ± 5 °C for 10 h, thawed in a refrigerator at 4 ± 2 °C for 2 h, and the obtained concentrated juice was collected. Subsequently, the concentrated reconstituted juice was prepared simulating a fast-food restaurant. Juice was reconstituted with each sample water (pure water, AlEW, and TMW) to obtain the °Brix value before concentration. The SOD activity, TSS, pH and sensory evaluation of the juices were analyzed.

Results

The pH difference and the characteristic changes of AlEW were analyzed by glass electrode pH meter and colorimetric method. The resulted showed that NaCl had no significant effect on the GpH and colorimetric pH of AlEW. Due to the presence of bubbles, the colorimetric reaction was inhibited and the contact between the water and the glass electrode was hindered. As a result, differences in pH values were created and the GpH and colorimetric pH measurements were affected, however independent of the type of bubbles. In addition, the colorimetric pH is closer to the ionic pH than the GpH value. When the colorimetric pH or GpH is constant, the theoretical pH of AlEW is greater than the ionic pH of the pH-adjusted water. Which means that the dissociation of AlEW can be promoted by electrolysis, thus increasing the content of $[OH^-]$ ions in AlEW. Hanaoka reported the reason of AlEW improved the antioxidant activity might be due to the increase of the ionic product of water as solvent. Therefore, it can be inferred that AlEW as a solvent can promote the dissociation of AsA and thus the antioxidant effect of AsA can be exerted to a greater extent.

Further, the influence of constituent factors of AlEW on antioxidant activity was also analyzed. The results showed that NaCl, NaOH and H_2 bubble were used as sample water regulators. And one-way analysis was performed, which showed that neither EC nor pH had a linear relationship with the SOD activity of the adjusted water. The results also showed that the pH, EC and hydrogen bubbles of HB-AW were the same as those of AlEW, but the SOD activity was almost the same as that of PW. Moreover, AlEW and TMW can significantly improve the SOD activity of AsA, which reached a maximum at an electrolysis current of 8A or a tourmaline stone treatment temperature of 75°C. The antioxidant activity of ASA was increased by 22.3% and 26.9% by AlEW and TMW, respectively. Furthermore, AlEW-SIW and TMW-SIW could improve the antioxidant activity of AsA.

And then, the application of functional water in concentrated fruit juice was studied. The SOD activity of juice reached the maximum value when the electrolysis current of AlEW was 8 A or tourmaline stone treatment temperature of TMW was 75 °C, which were determined as the reconstitution conditions of the concentrated juice. The results also showed that the SOD activity of 100% Tropicana concentrated reconstituted orange juice of the AlEW (77.2%) and TMW (84.5%) groups in-creased by 7.8 and 15.1%, respectively, compared with that of the control group (69.4%). The SOD activity of freshly squeezed orange juice increased by 7.9% and 7.7%, respectively. Tropicana apple juice and freshly squeezed apple juice SOD activity increased by more than 5.4% and 2.2%, respectively. In addition, no significant color difference was observed between the concentrated reconstituted juice of functional water and unconcentrated group. And functional water could improve the taste of juices. In summary, AlEW and TMW improved the antioxidant activity of the antioxidants in the concentrated juice.

Conclusion

Colorimetric pH (pH measured by colorimetric method) can be approximated by ionic pH (pH calculated from ionic concentration) while GpH has deviation from ionic pH. By comparing the glass electrode pH meter and the colorimetric method, it could be inferred that electrolysis promoted the dissociation of AlEW and increased the OH- ion content. It was indicated that, as a solvent, the water itself was affected by the electrical treatment. Physicochemical parameters of AlEW, pH (NaOH), EC (NaCl) and ORP (hydrogen bubbles) were not the main factors for AlEW to improve the SOD activity of ascorbic acid (AsA). AlEW at electrolytic currents from 4 to 16 A and TMW at tourmaline stone treatment temperatures from 25 to 100 °C showed significant ascorbic acid antioxidant improvement. The peak was reached at AlEW with an electrolytic current of 8A (89.67%) or TMW (94.25%) with a tourmaline stone treatment temperature of 75°C. AlEW and TMW, two types of functional water, can improve the antioxidant activity, increase sweetness, maintain sourness, and improve taste of juices. AlEW-SIW and TMW-SIW generated by applying microcurrent to pure silver electrodes with AlEW and TMW as electrolytes can obviously improve the antioxidant activity of AsA.

In summary, functional water, especially those containing AlEW and TMW, can improve the antioxidant activity of antioxidant substances and be used as a technique to extend the shelf life of products when used in beverages, which is beneficial to health as daily drinking water for long-term consumed.