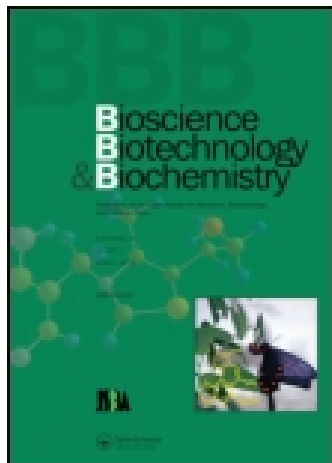


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Decomposition of Ethylene, a Flower-senescence Hormone, with Electrolyzed Anode Water

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Electrolyzed anode water (EAW) markedly extended the vase life of cut carnation flowers. Therefore, a flower-senescence hormone involving ethylene decomposition by EAW with potassium chloride as an electrolyte was investigated. Ethylene was added externally to EAW, and the reaction between ethylene and the available chlorine in EAW was examined. EAW had a low pH value (2.5), a high concentration of dissolved oxygen, and extremely high redox potential (19.2 mg/l and 1323 mV, respectively) when available chlorine was at a concentration of about 620 μ M. The addition of ethylene to EAW led to ethylene decomposition, and an equimolar amount of ethylene chlorohydrine with available chlorine was produced. The ethylene chlorohydrine production was greatly affected by the pH value (pH 2.5, 5.0 and 10.0 were tested), and was faster in an acidic solution. Ethylene chlorohydrine was not produced after ethylene had been added to EAW at pH 2.6 when available chlorine was absent, but was produced after potassium hypochlorite had been added to such EAW. The effect of the pH value of EAW on the vase life of cut carnations was compatible with the decomposition rate of ethylene in EAW of the same pH value.

These results suggest that the effect of EAW on the vase life of cut carnations was due to the decomposition of ethylene to ethylene chlorohydrine by chlorine from chlorine compounds.

Key words: electrolyzed anode water (EAW); ethylene decomposition; ethylene chlorohydrine; chlorine

Electrolyzed water having a sterilizing effect is roughly classified into two types: one is EAW (anode water of approximately pH 2.5 produced by diaphragm-type electrolyzing devices), and the other is weakly acidic or alkaline electrolyzed water (water of pH *ca.* 6–8 produced by non-diaphragm-type electrolyzing devices). These two types of electrolyzed water possess a similar sterilizing effect and antibacterial spectrum, and the difference between the two

types, except for the pH value, has not been clarified.^{1–3)} There is little information indicating the characteristics of electrolyzed water apart from its sterilizing effects.

The usefulness of electrolyzed water in medical and agricultural fields has recently been examined.^{4–6)} We have reported that, when used for flower arrangement, EAW could extend the vase life of cut roses because of its bactericidal effects from the low pH value and such chlorine compounds as hypochlorous acid.⁷⁾

In present study, we directed our attention to ethylene, one of the plant hormones that causes senescence of flowers and ripening of fruits.⁸⁾ It is well known that cut carnation flowers suffer from enhanced ethylene production and respiration rate associated with the onset of petal senescence.^{9–11)} We report here the effect of EAW on the vase life of cut carnations and the characteristics of EAW relevant to the decomposition of ethylene.

Materials and Methods

Reagents. Potassium chloride (KCl), potassium hydroxide (KOH), hydrochloric acid (HCl), sodium hydroxide (NaOH), sulfuric acid (H₂SO₄), ethylene glycol and ethylene chlorohydrine were of reagent grade and purchased from Kanto Chemical Co. (Tokyo, Japan). A potassium hypochlorite (KClO) solution (5–7%, laboratory grade) was purchased from Kanto Chemical Co., and 99.5% purity standard ethylene gas was obtained from Nippon Sanso Co. (Tokyo, Japan).

Production of electrolyzed anode water. Tap water for electrolysis was passed through an automatic water softener (SAT255A, Japan Organo Co., Tokyo, Japan), ion-exchange resin (G-35B, Japan Organo Co.), and Millipore filter (type N, Japan Organo Co.). This purified tap water was electrolyzed by the addition of potassium chloride (3.5 M starting concentration) at the rate of 7.7 ml/min with

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Abbreviation: EAW, electrolyzed anode water

a flow type of diaphragm electrolyzing device (Oasys Bio OW-01, Asahi Glass Engineering Co., Chiba, Japan). Both the anode and cathode water was produced at the rate of 1.04–1.06 l/min; the water used in the experiments was collected 5 min after electrolysis had been started, unless otherwise noted.

EAW was put into a plastic bottle (1 liter), corked lightly, and kept at room temperature for 20 days in the shade until available chlorine could no longer be detected. This dechlorinated EAW was also used to prepare a KClO solution.

Characteristics of EAW. The pH value, electrical conductivity, redox potential and dissolved oxygen were respectively measured with a pH meter (F-22, Horiba, Kyoto, Japan), electrical conductivity meter (CM-14P, Toa Electronics, Tokyo, Japan), redox potentiometer (RM-12P, Toa Electronics) and dissolved-oxygen meter (DO-21P, Toa Electronics) at 25°C.

The concentrations of potassium and chloride ions were respectively assayed with an atomic absorption spectrometer (Aanalyst 100, Perkin Elmer, Norwalk, CT, U.S.A.) and an ion chromatograph (DX-120, Dionex, Sunnyvale, CA, U.S.A.). The ion chromatograph was used under the following conditions: the column was a Dionex AS-4A type (4φ × 250 mm); the eluent was 1.8 mM Na₂CO₃/1.7 mM NaHCO₃; the flow rate was 1.5 ml/min; the column was used at room temperature; detection was by an EC detector; and the sample volume was 50 μl. The concentration of available chlorine in EAW was measured by iodometric titration.¹²⁾

Effect of EAW on vase life of cut carnations. Carnations (*Dianthus caryophyllus* L.) were obtained from a flower market on April 10, 2002. These flowers had not been treated with any preservatives and were purchased within 48 hours of harvesting. Stems were shortened to 40 cm, and twenty flowers each were placed in plastic bottles (4 liters) containing each vase solution. The flowers were maintained in a growth chamber under white fluorescent light (60 μmol/m²/s, 12 hours/day) at 25°C (light)–20°C (dark) and approximately 60% relative humidity. The vase solutions were EAW (original solution at pH 2.5), EAW at pH 5.0 or 10.0 adjusted with a small volume of 1N KOH, EAW without available chlorine, and ion-exchanged water. The vase life of the flowers was evaluated for symptoms of senescence involving inrolling of the petals.¹³⁾

Ethylene decomposition with EAW. Three hundred milliliters each of the following solutions was prepared: a) EAW (original solution at pH 2.5); b) EAW at pH 5.0 or 10.0 adjusted with a small volume of 1 N KOH; c) KClO solutions dissolved in EAW without available chlorine with the same concentra-

tion of available chlorine (620 μM) as EAW whose pH value had been adjusted to 2.5, 5.0 or 10.0 with a small volume of 1 N HCl or 0.1 N KOH; and d) EAW without available chlorine at pH 2.6 adjusted to pH 5.0 or 10.0 with a small volume of 1 N KOH. Each solution in a beaker was placed in a desiccator of approximately 4.5 liters volume. Standard ethylene gas (the final concentration was about 3 ppm) was then added to the desiccator. Thereafter, after 1 hour, and 1, 2 and 3 days, 2 ml of the gaseous phase was collected with a gas-tight syringe through a fluorine-containing rubber stopper on the side of the desiccator. The concentration of each ethylene sample was measured with a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan). The desiccator was placed in a room controlled to 25°C under 7–8 μmol/m²/s of irradiation (12 hr/day) or in the shaded condition.

The analytical condition used for GC were as follows: detection was by FID; the column was of stainless steel (3 mmφ × 3 m) loaded with Porapack Q (50/80 mesh); the column temperature was 60°C; the detector temperature was 100°C; the flow rate of carrier gas (N₂) was 50 ml/min.

Decomposition products of ethylene. EAW solutions a), b), c) and d) were prepared as just described. Five milliliters of each solution was put into a test tube of 12 mm diameter, and the test tube was ventilated with 10 ml of the standard ethylene gas through a gas-tight syringe for 2 min. Ethylene chlorohydrine and ethylene glycol as the decomposition products in each solution were measured with gas chromatograph from 1 to 180 min after the ventilation.

Solutions a) and c) were diluted by 2 to 8 times with EAW without available chlorine. The pH of each solution was adjusted to 2.5 with a small volume of 1 N HCl, and the available chlorine in each solution was measured. Standard ethylene gas was ventilated into each solution in the same manner as that just described. Ethylene chlorohydrine was measured with a gas chromatograph 1 min after the ventilation.

To confirm whether or not the single peak on the gas chromatogram could be identified as ethylene chlorohydrine, the following experiment was performed.¹⁴⁾ EAW (pH 2.5, 5 ml), in which ethylene chlorohydrine had been produced by ethylene ventilation, was mixed with 0.5 N NaOH (100 μl), held at 90°C for 30 minutes, and finally hydrolyzed with 36 N H₂SO₄ (15 μl). The resulting sample was assayed with a chromatograph under the following analytical conditions: detection by FID; glass column (2.6φ × 1 m) loaded with Unicarbon A-500 (80/100 mesh); column temperature of 115°C; detector temperature of 150°C; flow rate of carrier gas (N₂) of 20 ml/min.

pH effect of EAW on ethylene decomposition. EAW was adjusted in the pH range of 3.0 to 10.0

Table 1. Chemical Characteristics of Electrolyzed Anode and Cathode Water

	pH	EC (nS/m)	RP (mV)	DO (mg/l)	K ⁺ (mM)	Cl ⁻ (mM)	Available chlorine (μM as Cl ₂)
Anode water	2.53 \pm 0.04	597 \pm 25	1323 \pm 5	19.21 \pm 0.43	6.38 \pm 0.16	10.22 \pm 0.53	620 \pm 13
Cathode water	11.68 \pm 0.05	667 \pm 21	-646 \pm 5	1.86 \pm 0.11	13.22 \pm 0.18	10.52 \pm 0.46	ND

The applied potential was 12.5 V and the electrolytic current was 11.4 A. Each result is shown as the mean \pm S.E. of three determinations. EC, electric conductivity; RP, redox potential; DO, dissolved oxygen; ND, not determined.

with a small volume of 1 N KOH. Five-milliliter aliquots of these solutions were each put into a test tube with a diameter of 12 mm, and standard ethylene gas was ventilated into each solution in the manner already described. The production of ethylene chlorohydrine in each test tube was measured by gas chromatography 1 min after the ventilation. Diluted EAW and ethylene gas were also used for a similar experiment. EAW was double-diluted with EAW without available chlorine, and the solution was adjusted in the pH range of 3.0 to 10.0 with a small volume of 1 N KOH. Aliquots (5 ml) of these solutions were each put into a test tube, and 10 ml of the standard ethylene gas diluted five-fold with air was ventilated in the manner already described. Ethylene chlorohydrine was measured by gas chromatography 1 min after the ventilation. Standard ethylene chlorohydrine (750 μM final concentration) and ethylene glycol (900 μM final concentration) were dissolved in EAW without available chlorine. The analytical conditions used were the same as those already mentioned.

Results

Characteristics of EAW

As shown in Table 1, the pH value of EAW was 2.53, the redox potential was 1,323 mV, and dissolved oxygen was 19.21 mg/l. These values were higher than those for cathode water (-646 mV and 1.86 mg/l, respectively) and tap water before electrolysis (ca. 450 mV and 9.2 mg/l, respectively). The potassium and chloride ion concentrations were 6.4 mM and 10.2 mM, respectively. The available chlorine concentration measured by iodometry was 620 μM . EAW without available chlorine had a pH value of 2.6, close to that of EAW.

pH effect of EAW on the vase life of cut carnations

The vase life of cut carnations in EAW at different pH values was examined. The vase life in EAW was found to be affected by the pH value (Fig. 1). EAW (original solution at pH 2.5) gave a significantly longer vase life than EAW adjusted to pH 5.0 or 10.0. The vase life in EAW without available chlorine was almost the same as that in EAW of pH 10.0.

Ethylene decomposition with EAW

The change in concentration of ethylene in the

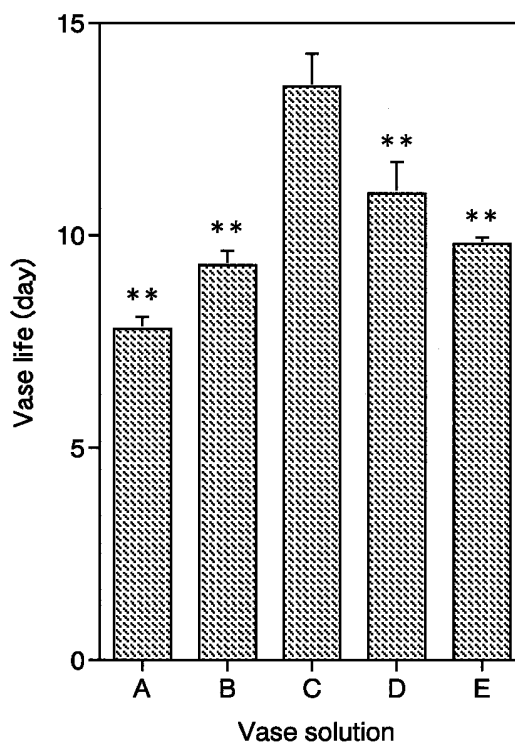


Fig. 1. Vase Life of Cut Carnations in Ion Exchanged Water (A), EAW without Available Chlorine (B), EAW (original solution, pH 2.5, C) and EAW with pH Adjusted to 5.0 (D) and 10.0 (E) with a Small Volume of 1 N KOH.

Twenty flowers each were placed into plastic bottles (4 liters) containing each vase solution. The flowers were maintained in a growth chamber under white fluorescent light (60 $\mu\text{mol}/\text{m}^2/\text{s}$, 12 hours/day) at 25°C (light) and 20°C (dark) and approximately 60% relative humidity. Each value is the mean \pm S.E. ** indicates significant difference ($p < 0.01$) from the value for flowers in EAW.

gaseous phase was examined in the presence of EAW in a closed system. The concentration of ethylene was slightly lower in the presence of EAW without available chlorine, while in the presence of EAW (pH 2.5), no ethylene could be detected 1 day after sealing the system. Ethylene was also not detected in the presence of EAW with the pH value adjusted to 5.0 3 days after sealing, although in the presence of EAW with the pH value adjusted to 10.0, approximately 80% of the ethylene remained after 3 days, similar to the result for EAW without available chlorine (pH 2.6) (Fig. 2). The KClO solutions with 620 μM of available chlorine in each and the pH value adjusted to 2.5, 5.0, or 10.0 showed similar results to EAW

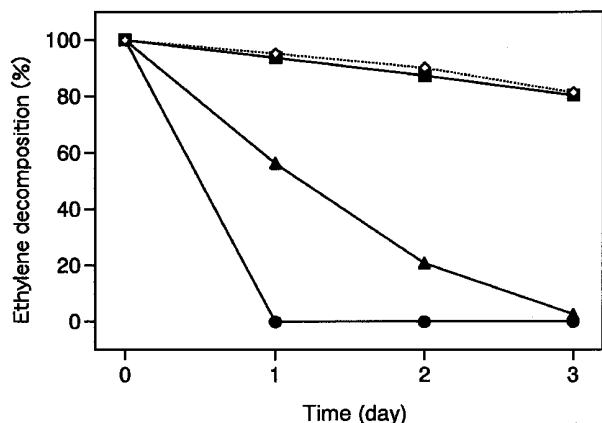


Fig. 2. Time-course Characteristics of Ethylene Decomposition in the Presence of EAW (original solution, pH 2.5, ●), EAW with pH Adjusted to 5.0 (▲) and 10.0 (■) with a Small Volume of 1 N KOH, and EAW without Available Chlorine (pH 2.6, ◇).

Three hundred milliliters of each solution in a beaker was placed in a desiccator of approximately 4.5 liters in volume. Standard ethylene gas was injected into the desiccator, the final concentration of ethylene being about 3 ppm. The temperature and irradiation were 25°C and 7–8 $\mu\text{mol}/\text{m}^2/\text{s}$ (12 hr/day), respectively. Two milliliters of the gaseous phase was collected with a gas-tight syringe through the rubber stopper on the side of the desiccator 1 hr, and 1, 2 and 3 days after ethylene injection. The ethylene concentration was measured by gas chromatography.

adjusted to these pH values, and EAW without available chlorine with the pH value adjusted to 5.0 or 10.0 produced similar results to EAW without available chlorine (pH 2.6) (data not shown). Similar results were obtained under the shaded condition (data not shown).

Decomposition products of ethylene

Ethylene was ventilated into EAW to determine its decomposition products. A gas chromatogram of standard ethylene chlorohydrine and ethylene glycol dissolved in EAW without available chlorine was obtained. Under these analytical conditions, a peak of ethylene chlorohydrine was detected at approximately 3 min, and a peak of ethylene glycol at approximately 15 min. A peak which was neither ethylene chlorohydrine nor ethylene glycol was detected when ethylene was ventilated into EAW without available chlorine. In respect of EAW, a peak corresponding to ethylene chlorohydrine was detected at approximately 3 min, while no peak of ethylene glycol could be detected. The chromatogram for the conversion of ethylene chlorohydrine showed no peak of ethylene chlorohydrine, but instead a peak of approximately equimolar concentration of ethylene glycol (data not shown).

pH effect of EAW on ethylene decomposition

The pH dependence of the ethylene decomposition rate in EAW, and the relationship between the con-

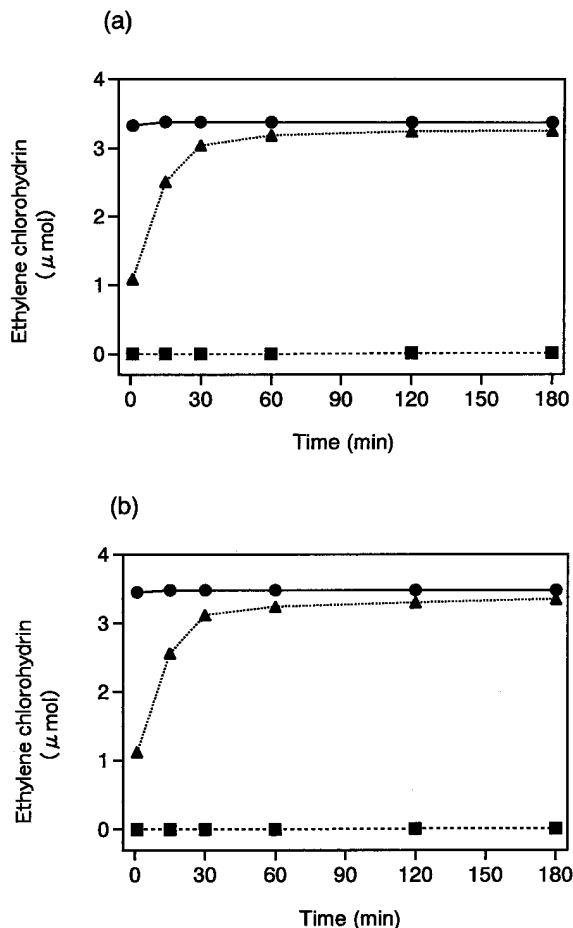


Fig. 3. Time-course Characteristics of Ethylene Chlorohydrin Production in EAW (a) and in KClO Solutions with the Same Available Chlorine as EAW (b).

The pH values of the solutions were 2.5 (●), 5.0 (▲) and 10.0 (■). A KClO solution was prepared with EAW without available chlorine, the available chlorine concentration of each solution being 620 μM . Ethylene chlorohydrin was measured 1, 15, 30, 60, 120 and 180 min after standard ethylene gas had been injected.

centration of available chlorine and the formation of ethylene chlorohydrine were examined in the reaction of EAW with ethylene in the liquid phase. As shown in Fig. 3(a), in EAW of pH 2.5, about 3.3 μmol of ethylene chlorohydrine was detected 1 min after ethylene ventilation, and no change in the amount produced was apparent up to 180 min after ventilation. In EAW of pH 5.0, the production of ethylene chlorohydrine was initially small at approximately 1 μmol 1 min after ethylene ventilation, but the production after 60 min had increased to 3.2 μmol , a similar value to that from EAW of pH 2.5. In contrast, no ethylene chlorohydrine was detected up to 180 min after ventilation in EAW of pH 10.0. In the KClO solutions with 620 μM of available chlorine (pH 2.5, 5.0 and 10.0), the results were similar to those in EAW (Fig. 3(b)). No ethylene chlorohydrine could be detected at any pH value in EAW without available

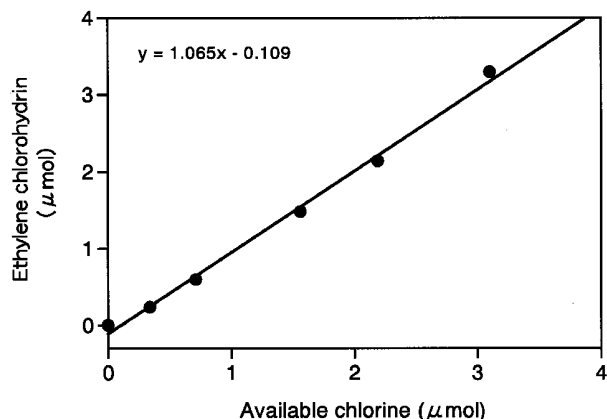


Fig. 4. Relationship between Available Chlorine and Ethylene Chlorohydrin Production in EAW (pH 2.5).

EAW was diluted to between 2 and 8 times with EAW without available chlorine, and the amount of ethylene chlorohydrin was measured 1 min after standard ethylene gas had been injected.

chlorine after being ventilated with ethylene (data not shown).

In EAW at pH 2.5, the correlation between available chlorine and the production of ethylene chlorohydrine 1 min after ventilation with ethylene is shown in Fig. 4. An equimolar amount of ethylene chlorohydrine to the available chlorine was detected. In the KClO solution with 620 μM of available chlorine (pH 2.5), a similar result to that in EAW was obtained (data not shown).

Almost the same amount of ethylene chlorohydrine 1 min after ethylene ventilation was produced in EAW in the pH range of 2.5 to 4, but the amount of ethylene chlorohydrine was less at pH 4.5 and higher. This production was particularly low in alkaline solutions (Fig. 5(a)). In EAW diluted twice with EAW without available chlorine, the production of ethylene chlorohydrine after ventilation with ethylene diluted 5-fold with air was varied between pH 2.5 and 4, a larger amount of ethylene chlorohydrine being produced in the more acidic solutions (Fig. 5(b)).

Discussion

Chloride ions are converted to chlorine (Cl_2) near the anode during electrolysis, and when Cl_2 dissolves in water, it is rapidly hydrolyzed to form available chlorine.¹⁵⁻¹⁷ The addition of KClO to water also produces chlorine.¹⁸ The available chlorine quoted in this work is the total of Cl_2 , hypochlorous acid (HClO) and hypochlorous acid ion (ClO^-). Their concentrations depend on the pH value when chlorine is dissolved, and equilibrium can be described as follows:¹⁶⁻¹⁸

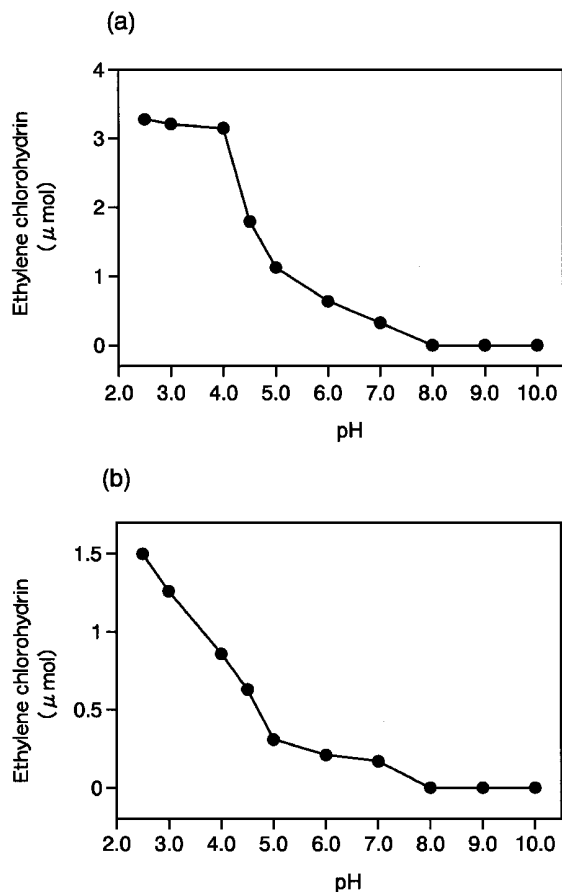
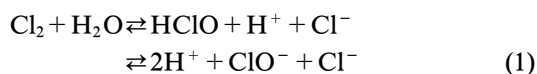


Fig. 5. Effect of pH Value on the Ethylene Chlorohydrin Produced in EAW without (a), and with (b) Diluted EAW (double) and Standard Ethylene Gas (5-fold).

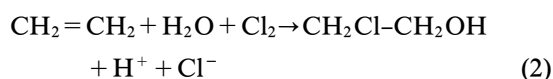
EAW was double-diluted with EAW without available chlorine, and standard ethylene gas was diluted 5-fold with air. The pH value of each solution was adjusted with a small volume of 1 N KOH, and ethylene chlorohydrin was measured 1 min after standard ethylene gas had been injected.

The available chlorine is composed of Cl_2 and HClO at a ratio of 1:9 in EAW at or near pH 2.5, is mostly of HClO at or near pH 5.0, and is of ClO^- only at or near pH 10.0.^{15,16,18,19}

It has been well established that the onset of senescence in cut carnations (*Dianthus caryophyllus* L.) is accompanied by a marked increase in the synthesis of ethylene, and a concomitant climacteric rise in respiration.⁹⁻¹¹ It has been reported that cut carnations at 20-24°C began producing a detectable increase in ethylene beginning 4-5 days after harvest and peaking at 6 days.^{20,21} Furthermore, treatment of presenescent flowers with ethylene has induced premature petal senescence.¹³ Pretreating carnations with inhibitors of ethylene synthesis or ethylene action such as the silver thiosulfate complex has prevented typical petal senescence.^{22,23} Figure 1 shows the pH dependence of vase life of carnations in EAW, the vase life being markedly longer in EAW of pH 2.5. This result is compatible with the ethylene

decomposition rate in EAW of the same pH values. It is therefore considered that EAW at pH 2.5 delayed the senescence of carnations by preventing an accumulation of ethylene.

Ethylene was decomposed in the presence of available chlorine in the basic solutions (Fig. 2), being decomposed to ethylene chlorohydrine in EAW, and the rate of ethylene chlorohydrine production was markedly lower in EAW at pH 5 or more (Fig. 5(b)). These results suggest that the main available chlorine involved in the decomposition of ethylene was probably Cl_2 , and that ethylene was broken down through the following reaction:



In EAW at or near pH 2.5, since ethylene reacts with Cl_2 , the equilibrium in reaction 1 is inclined toward the left. Therefore, Cl_2 was probably steadily supplied from HClO , and ethylene chlorohydrine equimolar to the available chlorine was finally produced. A gradual production of ethylene chlorohydrine was observed after ethylene ventilation at pH 5.0 (Fig. 3). This is considered to have been caused by the reaction of HClO with residual ethylene dissolved in EAW through following reaction 3, the second-order rate constant of this reaction probably being smaller than that of reaction 2:



As shown in Fig. 5(a), the amount of ethylene chlorohydrine produced 1 min after ethylene ventilation was no different between EAW at pH 2.5 and 4.0, but the decomposition rate of ethylene to ethylene chlorohydrine was faster at pH 2.5 than that at pH 4.0 when EAW had been diluted twice to reduce the available chlorine and the ethylene concentration had also been reduced to one-fifth. It is considered that the reaction between ethylene and Cl_2 was faster and completed within 1 min when there was no dilution of EAW and ethylene.

Recent reports about the sterilizing effect of electrolyzed water have demonstrated that the sterilizing activity of electrolyzed water at or near pH 2.5 was equal to that of acidic or alkaline water (pH ca. 6–8),^{1,2)} although these studies did not distinguish the usefulness between the two kinds of electrolyzed water. The results of the present study indicate that the effect of these two kinds of electrolyzed water on the vase life of cut carnations and the decomposition rate of ethylene is distinct.

EAW might influence ethylene synthesis as well as the decomposition of ethylene generated by carnation flowers. Further study is therefore necessary to fully elucidate the effect of EAW on plants.

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