Studies on the Origin of Chloroform in Vegetables

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To study the origin of chloroform in vegetables, a simple and sensitive method was established using a gas chromatograph equipped with an electron capture detector. The method includes solvent trapping of chloroform, and gas chromatographic separation with a detection system. The average concentrations in vegetables, tea leaves and frozen vegetables were 30.9, 62.9 and 15.7 μ/kg, respectively. Tea leaves contained the highest amount, followed by vegetables. The former was 2 times the latter. There was no significant difference between chloroform levels in vegetables grown in tap water (chloroform : 19.7–25.0 μg/L) and those in boiled distilled water. On the other hand, there was a high correlation between the number of cultivation days and chloroform levels in vegetables grown in both tap water and boiled distilled water.

Key words —— chloroform, vegetable, tea leaves, frozen vegetable

INTRODUCTION

Chloroform is found in all chlorinated tap water. This compound is formed as a result of the reaction between chlorine and organic precursors, typically naturally occurring substances like humic acids.[1,2] A National Cancer Institute (NCI) report showed that chloroform caused cancer in rats and mice under laboratory test conditions.[3] Japanese law has defined regulations for chloroform content in tap water (< 0.06 mg/l). Chloroform is a widely used industrial chemical. Water is possibly now the major source of environmental exposure to chloroform.[4] Although information has been published describing chloroform levels in drinking water, little is known about its daily intake. To assess this, we studied the amount and origin of this compound in vegetables. Vegetables may absorb chloroform *via* water and air. In this study, we discuss the origin of this compound in vegetables.

MATERIALS AND METHODS

Apparatus —— The apparatus was assembled as shown in Fig. 1. Flask A is for purging. The sample trap tube (B) is cooled by ice. Purging nitrogen gas was bubbled through the bomb.

Materials —— Commercial vegetables were used. The standard chloroform test for water quality was used. All other chemicals were of reagent grade.

Procedure —— Vegetable samples were cleaned with chloroform-free distilled water and then homogenized, weighed and analyzed. Ten grams of each sample was mixed with 200 ml chloroform-free distilled water in the purge flask (A) equipped with an impinger (B) containing 10.0 ml *m*-xylene (Fig. 1). The mixture was heated gradually to approximately 80°C on a mantleheater over nitrogen gas for 70 min. The purged chloroform was trapped in *m*-xylene cooled by ice at 0°C. The separated *m*-xylene was made up to 10.0 ml with fresh *m*-xylene and subjected to gas chromatography (GC) to assay the concentration of chloroform. The assay was performed on a Shimadzu Model GC-14B gas chromatograph equipped with an electron capture detector, and a wide-bore capillary column (ULBON HR-52, 30 m × 0.53 mm i.d.) was used. The column and the injection port were operated at 75°C, and the detector at 130°C with 40 : 1 split ratio. The nitrogen carrier gas flow was 3.8 ml/min. The conditions for the control experiments were the same as the analytical method mentioned above, but without the vegetable sample. Gas chromatography mass spectrometry (GC-MS) was performed on a Shimadzu GC-MS 9100-MK gas chromatograph-mass spectrometer system.

Statistical Analysis —— Values are shown as means ± S.D. of four separate determinations. Data were

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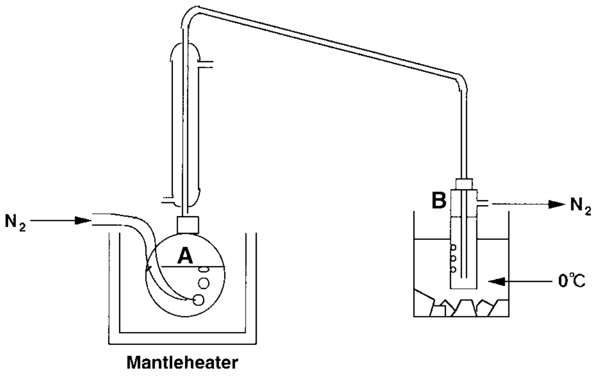

Fig. 1. Outline Figure of Apparatus for Distillation and Trap of Chloroform
A : purge flask, B : tap tube

analyzed using one-way analysis of variance (ANOVA) and, when appropriate, by a Student-Newman-Keul test. Results were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of bubbling time on the recovery of chloroform was studied using tomatoe, konacha and frozen pumpkin. An almost fixed recovery was obtained in the bubbling time over 30 min either sample. Therefore, the bubbling time in 70 min was adapted. The method was applied to the determination of chloroform in vegetable samples. The recoveries of added vegetable (30 μg/kg) in all the samples were 96.7–109.3%, with a maximum coefficient variation (c.v.) of 5.5%. The detection limit is 5 μg/kg.

The concentration of chloroform in vegetables, tea leaves and frozen vegetables was analyzed. Ichikawa[5] reported the formation of chloroform and carbon tetrachloride during the treatment of fresh vegetable homogenate with sodium hypochlorite. As chlorine reacts readily with various compounds,[1,6–10] it is possible that trihalomethanes are produced by the reaction of foods like fresh vegetables with sodium hypochlorite. Since sodium hypochlorite is used in the process of producing frozen vegetables,[5] frozen vegetables are also anticipated to contain chloroform. In vegetables, chloroform concentrations ranged from 9.8 to 135.7 μg/kg. Those in tea leaves and frozen vegetables were between 24.3 and 126.9 μg/kg, and between 9.3 and 25.6 μg/kg, respectively (Table 1). A peak corresponding to chloroform was observed in the chromatograms of the vegetable samples, and it was not disturbed by the other peaks (Fig. 2). This peak was confirmed as chloroform by GC mass spectrometry (Fig. 3). The average concentrations in vegetables, tea leaves and frozen vegetables were 30.9, 62.9 and 15.7 μg/kg, respectively (Table 2). When the mean values were compared,

Table 1. Concentration of Chloroform in Vegetables, Tea Leaves and Frozen Vegetables

Sample	Concentration (μg/kg)	Sample	Concentration (μg/kg)	Sample	Concentration (μg/kg)
Vegetables		Lettuce	17.4± 1.4	Genmaicha	54.1±2.4
Mitsuba	135.7±20.0	Chinese chive	17.3± 2.3	Uroncha 2	50.8±1.9
Cucumber	62.8± 6.1	Pumpkin	16.7± 5.1	Puarucha	46.9±0.3
Onion	50.0± 7.0	Shiitake	15.8± 1.7	Karigane	43.7±0.5
Broccoli	47.3± 4.8	Enokitake	14.7± 1.5	Aoyanagi	32.4±0.4
Welsh onion	46.0± 0.4	Root	14.2± 0.7	Ujicha	27.8±2.7
Cabbage	43.7± 5.2	Tomatoes	10.8± 1.7	Houjicha 2	26.2±1.9
Soybeen sprouts	33.8± 2.4	Daikon	9.8± 0.9	Konacha	24.3±1.3
Edible burdock	26.7± 2.6				
Carrot	25.7± 1.5	Tea leaves		Frozen vegetables	
Garland chrysanthemum	24.4± 2.8	Kukicha	126.9±37.9	Spinach	25.6±0.7
Green peas sprouts	21.0± 8.2	Kyobancha	106.9± 6.9	Green peas	22.4±1.5
Kaiware	20.8± 0.8	Uroncha 1	99.9±10.1	Mixed vegetables	14.3±0.8
Potatoes	20.7± 0.8	Hatomugicha	86.0± 6.5	Corn	14.2±0.7
Chinese cabbage	19.1± 7.9	Houjicha 1	80.8± 4.8	Pumpkin	14.0±3.4
Chingentsuai	18.3± 3.0	Habucha	79.0± 7.3	Kidney bean	13.6±1.9
		Jyo aoyanagi	58.4± 3.3	Broccoli	11.8±0.3
				Dasheen	9.3±0.4

Data represent the mean ± S.D. of four separate determinations.

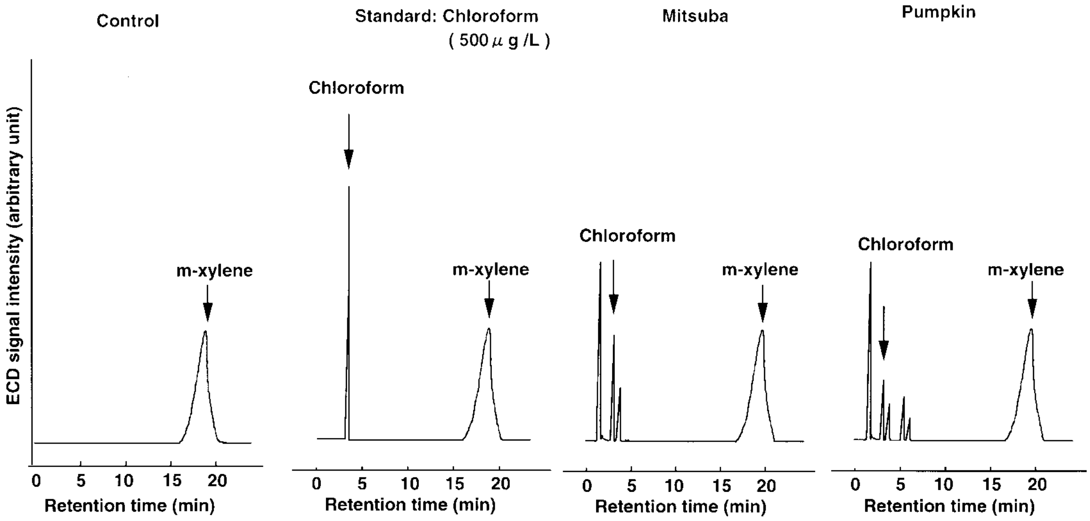

Fig. 2. Effect of Bubbling Time on the Recovery of Chloroform
—●— onion, —○— pumpkin, —△— Konacha, 0.3 μg of chloroform were added to 10 g each of onion, konacha and frozen vegetables (punpkin).

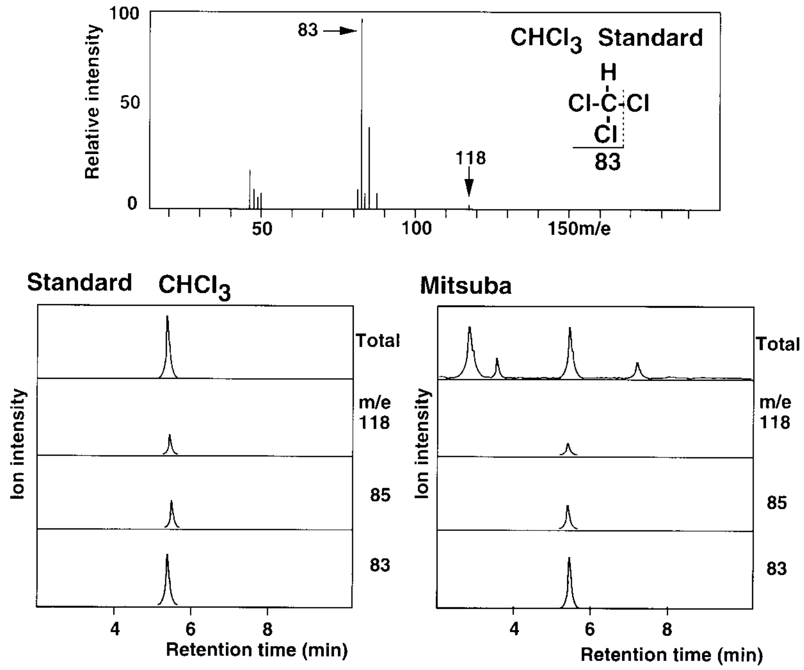

Fig. 3. Gas Chromatograms of Chloroform from Vegetables Trapped in *m*-xylene

tea leaves contained the highest amount followed by vegetables. The former was 2 times the latter. Unexpectedly, the chloroform content in the frozen vegetables showed the lowest value. Toyoda *et al.*[11] reported a chloroform content in mixed fruit drinks of 19.3 μg/kg. Their value is similar to that in frozen vegetables found here. The mixed fruit drinks may have been prepared from frozen fruit.

Next, we studied the origin of chloroform in

Table 2. Concentration of Chloroform in Vegetables, Tea Leaves and Frozen Vegetables

Sample	n	Concentration (μg/kg) Min–Max	Concentration (μg/kg) Mean ± S.D.$^{a)}$
Vegetables	23	8.2–135.7	30.9±26.9
Tea leaves	15	24.3–126.9	62.9±32.1
Frozen vegetables	8	9.3–25.6	15.7± 5.5

a) Data represent the mean ± S.D. of four separate determinations.

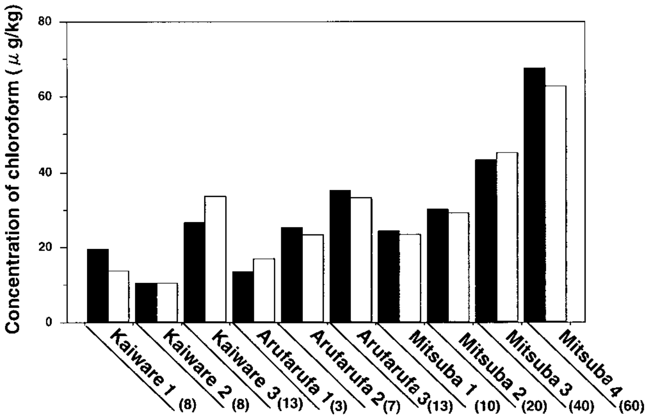

Fig. 4. Chloroform Levels in Vegetables Cultivated in Tap Water and in Boiling Distilled Water
▬ : Vegetables cultured in tap water (chloroform: 19.7 μg/kg). ▭ : Vegetables cultured in boiling distilled water. The numeral of the parenthesis shows the cultivation days.

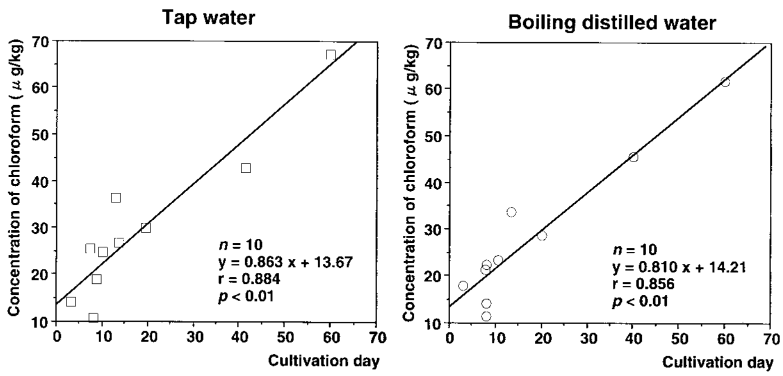

Fig. 5. Correlation between Cultivation Days and Chloroform Levels in Vegetables

vegetables. Generally, plants absorb organic compounds *via* the roots and accumulate these in the leaves.[12] Therefore, chloroform levels in vegetables grown in tap water (chloroform: 19.7–25.0 μg/l) and in boiled distilled water without chloroform were compared. Kaiware, alfalfa and mitsuba were used. The cultivation period for mitsuba is 10 days, 20 days, 40 days or 60 days. There was no significant difference in chloroform levels between the two water sources (Fig. 4). The relationship between cultivation days and chloroform content in vegetables was investigated. The chloroform content in vegetables increased with increasing cultivation days for vegetables grown in tap water and in boiled distilled water, and there was a high correlation found (Fig. 5). There are two routes for absorption of chloroform by vegetables, *i.e.*, *via* water and air. Toyoda *et al.*[11] showed the presence of chloroform in oils at levels of 4.9–38.5 ppb. They stated that chloroform in oils is derived from the atmosphere. The above findings show that the absorption of chloroform by leaves seems to surpass that from the root. The concentration of chloroform in indoor air during the experiment was 0.0011–0.0055 μg/l.

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