

## **Test 2 Confirmation Tests for Chemical Injury of 'Fuji' Apples in Practical Methyl Bromide Fumigation**

### **Materials and Methods**

#### **1. Test Fruit**

##### **'Bagged Fuji' Apples**

Medium size apples (36 per box) harvested on the 10th of November 1989 were obtained from a packing house in Hirosaki City, Aomori Prefecture. The fruit were stored at  $-1$  to  $0^{\circ}\text{C}$  for 30, 40, 70, 90 and 120 days under Standard Cold storage (SC storage) and for 120 days at  $-1$  to  $0^{\circ}\text{C}$  under Controlled Atmosphere storage (CA storage:  $\text{O}_2$  2%,  $\text{CO}_2$  2%). These apples were then maintained for 3 days at fumigation temperature of  $15^{\circ}\text{C}$  or  $10^{\circ}\text{C}$  prior to fumigation.

##### **'Unbagged Fuji' Apples**

Apples harvested on the 18th of November 1989 were obtained from a packing house in Kuroishi City, Aomori Prefecture. The fruit were stored at  $-1$  to  $0^{\circ}\text{C}$  for 30, 40, 70, 90 and 120 days under SC storage. These fruit were then maintained for 3 days at fumigation temperature of  $15^{\circ}\text{C}$  or  $10^{\circ}\text{C}$  prior to fumigation.

#### **2. Fumigation**

'Bagged and Unbagged Fuji' stored for a given period of time were placed in export carton boxes or plastic field bins in the following manner and these fruit were then placed in a  $0.52\text{ m}^3$  stainless steel fumigation chamber ( $0.9\text{ m} \times 0.66\text{ m} \times 0.86\text{ m}$  in size) equipped with  $0.86\text{ m}^3/\text{min}$ . circulation and ventilation apparatus, graduated dispenser, ampoule breaker and vaporizer for methyl bromide application, ports for gas sampling, and temperature probes and a manometer.

##### **Fruit Packed in Export Cartons**

Each apple was covered with a meshed polyethylene fruit cap and packed with two layers (36 fruit, ca. 10 kg) in the export carton ( $38\text{ cm} \times 44\text{ cm} \times 25\text{ cm}$  in size,  $0.043\text{ m}^3$  in capacity) with six fiberglass screen-covered vents (4 vents:  $2\text{ cm} \times 5\text{ cm}$ , 2 vents:  $4\text{ cm} \times 2\text{ cm}$ ; vent ratio of  $0.74\text{ m}^3$ ) on four sides. One macerated paper sheet was placed on the bottom of the carton and between the first and second layer, and one polyethylene meshed sheet was placed on the top of the first layer. The carton was sealed with sealing tape. These apples were stored for 3 days at fumigation temperature of  $15^{\circ}\text{C}$ . Five of the cartons (ca. 50 kg) were placed in a  $0.52\text{ m}^3$  fumigation chamber to make a load of 40% and fumigated for 2 hours at  $15^{\circ}\text{C}$  with doses of 30 or  $35\text{ g}/\text{m}^3$  of methyl bromide with 40% loading.

### **Fruit in Plastic or Wooden Field Bins**

A 0.062 m<sup>3</sup> plastic field bin with many vent holes or a wooden field bin (31.8 cm×63.5 cm×32 cm in size,) were used for fumigation. Seventy or 80 fruit (ca. 20 kg) were placed in both bins and stored for 3 days at fumigation temperature of 10°C. Four bins (ca. 80 kg) were placed in a 0.52 m<sup>3</sup> fumigation chamber to make a load of 50% were fumigated for 2 hours at 10°C with methyl bromide doses of 40, 45 or 50 g/m<sup>3</sup>.

Methyl bromide was applied by using a built-in ampoule breaker or a graduated dispenser. The built-in circulation apparatus was kept on for the first 30 minutes and an automatic timer was used (on : 0.5 minutes, off : 2.5 minutes) throughout the fumigation. Gas concentrations and air and fruit pulp temperatures during fumigation were monitored using a gas chromatograph (FID: GC 8AF, Shimazu) and a multi-channel automatic temperature recorder (Hybrid Recorder: AH, Chino). Following fumigation, the air-fumigant mixture was exhausted for one hour at 15°C or 10°C by using the built-in ventilation apparatus.

### **3. Storage of Fumigated Fruit**

Fumigated fruit were packed in export cartons and stored for 2 to 8 weeks at 0°C and then for 1 week at 15°C or 2 weeks at 20°C, which are conditions simulating commercial transport to the United States.

### **4. Evaluation of Injuries**

Fumigated fruit was evaluated mainly by visible observation of the symptoms on the skin and the pulp. However, firmness, soluble solids content and malic acid content of fruit fumigated at 15°C were assessed. Both sides of each fruit were cut in a circle approximately 2 cm in diameter and the firmness of fruit was measured using a fruit firmness tester (Magness-Taylor Type, Fujihira) with a 7/16-inch diameter plunger.

Samples for soluble solids content were obtained from the juice, which was made of the pulp free of the core portion. Soluble solids were measured with a temperature-compensated digital refractometer (PR-1, Atago). Malic acid content was determined by acid-base titration. A few drops of phenolphthalein was added to 5 mℓ of the solution made of the pulp. The amount of 0.1 N-NaOH used for the titration was calculated in terms of the amount of malic acid content in 100 mℓ of the solution.

## **Results and Discussion**

### **1. Gas Concentration During Fumigation**

Methyl bromide gas concentrations during fumigation were shown in Table 4-3 (fruit packed in export cartons) and Table 4-4 (fruit in plastic field bins and wooden bins), respectively. The average sorption ratio to initial doses of methyl bromide were 12.4%, 6.4% and 14.0%, respectively, for fruit packed in export cartons, those in plastic field bins, and in wooden bins.

**Table 4-3.** Methyl bromide concentrations recorded in a 0.52 m<sup>3</sup> fumigation chamber. Fumigation at doses of 30 g/m<sup>3</sup> and 35 g/m<sup>3</sup> for 2 hours at 15°C (Test 2).

Variety	Cold storage		Dose (g/m <sup>3</sup> )	Gas concentration (mg/ℓ)	
	Temperature (°C)	Period (Day)		30	120 min
Bagged	-1-0	SC 40	30	27.6	26.6
Fuji		SC 70	35	34.6	32.4
		SC120	35	32.5	29.8
		CA120	35	33.7	30.2
Unbagged	-1-0	SC 40	30	27.9	26.5
Fuji		SC 70	35	33.0	30.3
		SC120	35	32.3	30.0

**Table 4-4.** Methyl bromide concentrations recorded in a 0.52 m<sup>3</sup> fumigation chamber. Fumigation at doses of 40 g/m<sup>3</sup>, 45 g/m<sup>3</sup> and 50 g/m<sup>3</sup> for 2 hours at 10°C (Test 2).

Variety	Cold storage		Dose (g/m <sup>3</sup> )	Gas concentration (mg/ℓ)	
	Temperature (°C)	Period (Day)		30	120 min.
Bagged Fuji	-1-0	SC 30	40*	40.9	37.9
		SC 40	45**	41.6	38.5
		SC 70	50**	47.6	44.2
		SC 90	50**	49.2	41.6
		SC120	50*	50.4	46.7
		CA120	50*	51.1	46.5
Unbagged Fuji	-1-0	SC 30	40*	40.9	37.9
		SC 40	45**	42.2	38.7
		SC 70	50*	49.1	46.2
		SC 90	50**	45.3	42.5
		SC120	50**	50.0	43.8

\* Fruit in plastic field bins were fumigated

\*\* Fruit in wooden field bins were fumigated.

## 2. Fruit Injury

Results of the test are shown in Table 4-5 (Bagged Fuji) and Table 4-7 (Unbagged Fuji) for fruit packed in export cartons and fumigated at doses of 30 and 35 g/m<sup>3</sup> for 2 hours at 15°C, and Table 4-6 (Bagged Fuji) and Table 4-8 (Unbagged Fuji) for fruit in plastic or wooden field bins fumigated at doses of 40, 45 or 50 g/m<sup>3</sup> for 2 hours at 10°C.

### 'Bagged Fuji' Apples

No injury was observed on fruit fumigated at 15°C regardless of the length of the SC storage period prior to fumigation. Fruit stored for 120 days prior to fumigation and then stored in CA storage at 0°C for 6 weeks and at 20°C for 2 weeks showed a slight discoloration of the tissues of the core region. It was considered that this symptom was a storage injury which was generally observed on fruit stored at high temperatures or for long

**Table 4-5.** Phytotoxic responses of 'Bagged Fuji' apples packed in export cartons fumigated with methyl bromide at doses of 30 to 35 g/m<sup>3</sup> for 2 hours at 15°C with 40% loading (Standard 1) (Test 2).

Storage period & doses	Storage period after fumigation		No. of fruit injured/tested				Firmness (kg)		Soluble solids content (%)		Malic acid content (g/100 ml)	
			skin		pulp							
			Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.
SC -1-0°C	0°C 2 W	20°C 2 W	0/36	0/36	0/36	0/36	13.9	13.7	13.1	12.4	0.26	0.27
40 days	4	2	0/36	0/36	0/36	0/36	13.7	13.4	13.0	11.9	0.24	0.26
MB30 g/m <sup>3</sup>	6	2	0/36	0/36	0/36	0/36	13.2	13.7	12.9	12.3	0.24	0.24
SC -1-0°C	2	2	0/36	0/36	0/36	0/36	13.8	14.2	13.1	13.1	0.25	0.23
7 days	4	2	0/36	0/36	0/36	0/36	13.2	14.4	12.7	12.7	0.25	0.26
MB35 g/m <sup>3</sup>	6	2	0/36	0/36	0/36	0/36	13.0	13.7	13.0	13.0	0.27	0.21
SC -1-0°C	2	2	0/36	0/36	0/36	0/36	12.8	13.6	12.7	12.0	0.20	0.19
120 days	4	2	0/36	0/36	0/36	0/36	13.1	13.7	13.1	13.0	0.20	0.19
MB35 g/m <sup>3</sup>	6	2	0/36	0/36	0/36	0/36	13.1	13.5	13.2	13.0	0.18	0.17
CA -1-0°C	2	2	0/36	0/36	2/36	0/36	14.6	14.8	13.0	12.7	0.25	0.25
120 day	4	2	0/36	0/36	2/36	1/36	13.5	13.6	12.9	12.9	0.20	0.21
MB35 g/m <sup>3</sup>	6	2	0/36	0/36	2/36	0/36	13.4	13.0	12.8	12.8	0.25	0.19

**Table 4-6.** Phytotoxic responses of 'Bagged Fuji' apples placed in plastic bins fumigated with methyl bromide at doses of 40 to 50 g/m<sup>3</sup> for 2 hours at 10°C with 50% loading (Standard 2) (Test 2).

Storage period & dose	Storage period after fumigation		No. of fruit injured/tested			
			skin		pulp	
			Treat.	Cont.	Treat.	Cont.
SC -1-0°C	0°C 2 W	15°C 1 W	0/16	0/16	0/16	0/16
30 days						
40 g/m <sup>3</sup>						
SC -1-0°C	2	1	0/36	0/36	0/36	0/36
40 days	4	1	0/36	0/36	0/36	0/36
45 g/m <sup>3</sup>						
SC -1-0°C	2	1	0/18	0/18	0/18	0/18
70 days	4	1	0/18	0/18	0/18	0/18
50 g/m <sup>3</sup>	6	1	0/18	0/18	0/18	0/18
	8	1	0/18	0/18	0/18	0/18
SC -1-0°C	2	1	0/16	0/16	0/16	0/16
90 days						
50 g/m <sup>3</sup>						
SC -1-0°C	2	1	0/16	0/16	0/16	0/16
120 days	4	1	0/16	0/16	0/16	0/16
50 g/m <sup>3</sup>						
CA -1-0°C	2	1	0/16	0/16	0/16	0/16
120 days	4	1	0/16	0/16	0/16	0/16
50 g/m <sup>3</sup>	6	1	0/16	0/16	0/16	0/16

**Table 4-7.** Phytotoxic responses of 'Unbagged Fuji' apples packed in export cartons fumigated with methyl bromide at doses of 30 to 35 g/m<sup>3</sup> for 2 hours at 15°C with 41% loading (Standard 1) (Test 2).

Storage period & doses	Storage period after fumigation		No. of fruit injured/tested				Firmness (lbs)		Soluble solids content (%)		Malic acid content (g/100 mℓ)	
			skin		pulp		Treat.	Cont.	Treat.	Cont.	Treat.	Cont.
			Treat.	Cont.	Treat.	Cont.						
SC -1-0°C	0°C 2 W	20°C 2 W	0/36	0/36	0/36	0/36	14.0	13.8	14.6	14.6	0.25	0.27
40 days	4	2	0/36	0/36	0/36	0/36	13.8	13.6	14.4	14.4	0.24	0.26
MB30 g/m <sup>3</sup>	6	2	0/36	0/36	2/36	0/36	12.7	13.6	13.6	14.4	0.20	0.24
SC -1-0°C	2	2	0/36	0/36	0/36	1/36	13.1	14.0	13.2	14.2	0.25	0.23
70 days	4	2	0/36	0/36	0/36	0/36	12.8	13.3	15.2	14.0	0.22	0.20
MB35 g/m <sup>3</sup>	6	2	0/36	0/36	1/36	3/36	12.6	12.6	15.2	14.9	0.25	0.21
SC -1-0°C	2	2	0/36	0/36	0/36	0/36	13.1	12.9	15.4	14.7	0.20	0.19
120 days	4	2	0/36	0/36	2/36	1/36	13.1	12.7	14.7	14.1	0.18	0.16
MB35 g/m <sup>3</sup>	6	2	0/36	0/36	1/36	1/36	12.6	12.3	14.6	14.3	0.19	0.15

**Table 4-8.** Phytotoxic responses of 'Unbagged Fuji' apples placed in plastic bins fumigated with methyl bromide at doses of 40 to 50 g/m<sup>3</sup> for 2 hours at 10°C with 50% loading (Standard 2) (Test 2).

Storage period & dose	Storage period after fumigation		No. of fruit injured/tested				
			skin		pulp		
			Treat.	Cont.	Treat.	Cont.	
SC -1-0°C							
30 days	0°C 2 W	15°C 1 W	0/14	0/14	0/14	0/14	
MB40 g/m <sup>3</sup>							
SC -1-0°C	2	1	0/36	0/36	0/26	0/36	
40 days	4	1	0/36	0/36	0/36	0/36	
MB45 g/m <sup>3</sup>							
SC -1-0°C	2	1	0/18	0/18	0/18	0/18	
70 days	4	1	0/18	0/18	0/18	0/18	
MB50 g/m <sup>3</sup>							
SC -1-0°C	2	1	0/16	0/16	0/16	0/16	
90 days	4	1	0/16	0/16	0/16	0/16	
MB50 g/m <sup>3</sup>							
CA -1-0°C	2	1	0/16	0/16	3/16	2/16	
120 days	4	1	0/16	0/16	2/16	2/16	
MB50 g/m <sup>3</sup>							

storage periods, (KUDO, 1984 ; FUKUDA, 1985), because the symptom was observed on a part of unfumigated control fruit in this test.

As for firmness, soluble solids content and malic acid content, no differences were observed between the untreated fruit and the treated fruit at all doses of methyl bromide. No injury was observed on the skin or the pulp of fruit fumigated at 10°C of all doses of

methyl bromide regardless of the length of the storage period prior to fumigation.

### **'Unbagged Fuji' Apples**

In fruit fumigated at 15°C, injury to the skin and the pulp tended to increase with the duration of storage with both untreated and treated fruit.

As for firmness, soluble solids content and malic acid content, no differences were observed between fruit in the untreated lots and those in the treated lots at all doses of methyl bromide. In fruit fumigated at 10°C, a slight discoloration of the pulp was observed on both untreated and fumigated fruit stored for as long as 120 days. It could be said that these symptoms were not caused by methyl bromide fumigation, because the symptoms were very similar to those on fruit stored for long periods.

It could be concluded from these results that injuries by methyl bromide fumigation would not be observed on either 'Bagged or Unbagged Fuji' apples, which had been stored at -1 to 0°C for 30 days or more prior to fumigation, and that no injury caused by methyl bromide fumigation would be observed on fruit when 'Fuji' apples were disinfested with the combined cold storage and methyl bromide fumigation treatment established for export of apples to the United States.