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Some Problems with 1,2-Dibromoethane Residue Analysis

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Imported fruits were assayed for EDB residues. The relative standard deviations among nine individuals in each lot were 100 (max) and 41% (min) in mango and were 90 (max) and 27% (min) in papaya, respectively. Blending of the sample for 30 s for homogeneity caused about 11-15% of decreased EDB residue levels. The analytical results for grapefruit after separation into pulp, seed, and peel showed that EDB residue levels are very high in seed (2550 μg/kg (max) of sample), low in peel (3.97 μg/kg (max) of sample), and negligible in pulp (0.51 μg/kg (max) of sample).

Since the introduction of the Mediterranean fruit fly [Diptera, Tephritidae, *Ceratitis capitata* (Wiedemann)] into California in 1980, the quarantine treatment by 1,2-dibromoethane [commonly referred to as ethylene dibromide (EDB)] to citrus fruits, papaya, and mango, which are produced in the Trypetidae-occuring area, has been needed prior to its acceptance to Japan. However, EDB residues in those agricultural products had to meet strict legal tolerance requirement by the Ministry of Health and Welfare in Japan, depending on the recommendation of the FAO/WHO Joint Meeting (FAO/WHO, 1967) that no residue of EDB treatment be allowed to reach the consumer because of its carcinogenicity.

There are many reports on determination of EDB, and they have been summarized by Newsome and Papino (1977), King et al. (1980), and Rains and Holder (1981).

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In Japan a method using the combination of a distillation apparatus, which had been devised by Bielorai and Alumot (1966), and steam distillation with *n*-hexane has been used as a Japanese official method since 1981 (Sekita et al., 1983).

This paper deals with some problems on EDB analysis depending on the results obtained by applying this method to fruits.

EXPERIMENTAL SECTION

Reagents. EDB was a product of Tokyo Chemical Industry Co. Ltd. Pesticide-grade hexane was purchased from Wako Pure Chemicals Industry Co. and showed several peaks on the gas chromatogram after exposure to air or keeping EDB standard solutions in refrigerator but did not interfere with the analysis of EDB. Florisil PR was a product of Floridin Co. and was used after overnight activation at 130 °C. Silicone oil was a product of Shinetsu Chemical Co. Purified water was obtained by a double distillation, using all-glass apparatus, of tap water passed

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Table I. EDB Residues in Imported Grapefruit, Mango, and Papaya and Variation of Its Content among Individuals[a]

	μg of residues/kg of sample												
sample lot	1	2	3	4	5	6	7	8	9	mix-1[f]	mix-2[f]	mean[g]	max/min[h]
						Grapefruit[b]							
A	3.6	1.1	1.0	0.4	ND[i]	ND	ND	2.5	2.9	ND	ND	1.3	
B	0.8	1.7	ND	3.9	0.5	ND	0.1	4.0	7.4	2.7	ND	2.3	
						Mango							
A[c]	7.2	5.5	7.3	7.8	4.3	8.3	3.3	2.4	3.0	5.0	5.2	5.5	3.5
B[c]	41	16	18	27	155	11	72	85	9	41	42	48	17
C[c]	552	458	153	150	258	80	118	238	106	-[j]	-	235	6.9
						Papaya[e]							
A	108	90	98	83	98	158	164	102	92	-	-	110	2.0
B	71	21	30	69	94	33	224	24	66	-	-	70	11
C	16	128	33	37	20	140	26	24	131	-	-	62	6.6
D	19	24	38	36	24	23	9	23	65	-	-	29	7.2
E	72	16	45	16	46	8	25	112	15	-	-	39	14

[a] Each sample was collected at random at the airport or the port after the routine quarantine inspections during Dec 1986–May 1987 and assayed for EDB in the same day for each lot. [b] Imported from Florida, peel removed, and subjected to pretreatment A. [c] Imported from The Philippines, sliced to remove the seeds, and subjected to pretreatment A. [d] Imported from The Philippines, sliced to remove the seeds, and subjected to pretreatment B. [e] Imported from Hawaii and cut into wedge-shaped tetrasected portions. Their diagonal portions were collected, seeds were removed, and the samples were subjected to pretreatment B. [f] The same amount of each individual from sample 1–9 was collected, mixed, subjected to pretreatment A, and assayed for EDB, independent of individual assays. [g] Arithmetic means of the results of nine individuals. [h] Ratios of the maximum residue levels to minimum residue levels in each lot. [i] ND < 0.1 μg of residues/kg of sample. [j] Not determined.

through an ion-exchange column.

Apparatus. (*a*) *Gas Chromatograph.* A Shimadzu gas chromatograph, Model 9A, equipped with a ^{63}Ni electron-capture detector with a 2 m × 3 mm glass column packed with 20% Carbowax 20M on 80–100-mesh Gas Chrom Q (operating conditions: column flow, 80 mL/min N_2; column inlet, 250 °C; detector temperature, 250 °C; column temperature, 120 °C) or a 2 m × 3 mm glass column packed with 10% DC-200 on 80–100-mesh Chromosorb W (operating conditions: column flow, 30 mL/min N_2; column inlet, 250 °C; detector temperature, 250 °C; column temperature, 60 °C) was used. A Shimadzu C-R2AX was used for data treatment.

(*b*) *Distillation Apparatus.* The apparatus devised by Bielorai and Alumot (1966) was used with a 1-L distillation flask.

(*c*) *Filter Paper.* Whatman phase separators (silicone treated, 9 cm) were used.

(*d*) *Blender.* A Nihon Seiki Seisakusho Multi-blender Mill was used with a 500-mL cup.

Procedures. Principally the procedures used followed the method of Sekita et al. (1981). Pretreatment of fruits was carried out according to A and B. Pretreatment A: Analytical portions of fruits were cut into small pieces, put into a blender cup, and then blended for 30 s for homogeneity at room temperature at 5× (rpm) of a propeller blade and at 5× (rpm) of an eccentric spin of blender cup. Pretreatment B: Analytical portions of fruits were chopped into ≈1-cm cubes or squares. For a typical assay, a 50–100-g sample of grapefruit, mango, or papaya (grapefruit seeds 0.2–2 g) was weighed into a 1-L round-bottomed distillation flask. Then, 200 mL of water, 10 mL of *n*-hexane, two drops of silicone oil, and three to five pieces of boiling stone were added. After the apparatus was set up, sealing each joint with water and filling the receiver with water, the flasks were gently heated and the hexane was distilled with water for 1 h by use of heating mantles. After cooling, the collected hexane layer in the receiver was separated from the water layer by opening the glass cocks and then filtered through silicone-coated filter papers. The inside of the condenser and receiver was rinsed with a small amount of *n*-hexane, and the solution was again filtered and then combined with the previous *n*-hexane layer. After adjustment of the volume to 10 mL, about 1 mL of Florisil PR was added to the solutions and then the solution was vigorously shaken. After 15 min, the solution was diluted as necessary so the response would be within the linear range of the detector as determined by injecting a series of standard solutions (0.4 ng, at least 400 ng or more for DC-200 at retention time 6.0 min; 2 ng, at least 400 ng or more for Carbowax 20M at retention time 5.1 min). Usually 2 μL of the solution was injected in a GC column except 5 μL was injected for peel and pulp of grapefruit.

RESULTS AND DISCUSSION

Table I shows the analytical results of EDB contents in individual grapefruits, mangos, and papayas, which were imported from two countries and sampled at random immediately after passing through customs. Usually grapefruit shipped from Florida to Japan is in transit for 3–4 weeks. Papaya and mango are carried by plane from Hawaii and The Philippines, respectively. As it is well-known that EDB residue level in fruits decreases very rapidly with time (King et al., 1980; Sekita et al., 1983), the assays were performed on the day of arrival.

There were high variations in EDB contents in mangos and papayas of each lot. Especially lot B in mango and lot E in papaya showed residue ratios of maximum to minimum, about 17 and 14, respectively. This means great care must be taken to the number of samples representing a lot.

In mango the values obtained from the mixed mangos showed good agreement with the mean values of each mango as expected, but those of grapefruit did not and even ND values were observed. On the other hand, the relative standard deviations when 2 and 5 μL of EDB solution were injected in the gas chromatograph were 1.2 and 1.0% (n = 10), respectively and these values are very small. In addition to this, the recoveries (mean ± SD, n = 4) of EDB added to water, mango, papaya, and grapefruit in distillation flasks at levels of 2 and 20 μg of EDB/kg of samples were 79.6 ± 1.1 and 79.7 ± 0.6, 76.3 ± 3.0 and 82.6 ± 2.9, 79.0 ± 1.4 and 79.9 ± 1.7, and 79.6 ± 2.3 and 82.8 ± 1.5%, respectively. This indicates that the presented analytical method has enough accuracy for the present discussion. Therefore, the unexpected results on grapefruit confused us and brought us to reconsider the analytical method of EDB. First, we considered the big

Table II. Effect of Sample Pretreatment on Analytical Results of Mango, Papaya, and Grapefruit[a]

	μg of residues/kg of sample										
pretreatment[b]	1	2	3	4	5	6	7	8	9	10	mean
					Mango[c]						
A	44.0	37.2	36.8	38.4	35.2	53.2	16.6	34.4	52.8		
B	48.8	46.0	38.5	44.0	38.4	61.6	19.0	40.0	58.8		
B/A[e]**	1.11	1.24	1.05	1.16	1.09	1.16	1.14	1.16	1.11		1.13
					Papaya[c]						
A	124	98.9	87.5	164	113	30.2	22.6	35.1	36.4	37.1	
B	141	93.9	95.4	179	145	32.5	27.9	35.1	41.5	42.0	
B/A[e]***	1.13	0.95	1.09	1.09	1.28	1.07	1.23	1.00	1.14	1.13	1.11
					Grapefruit[c]						
A	7.2	2.9	5.0	ND[d]	3.9	4.0	ND	2.8	2.4	2.5	
B	7.5	3.5	ND	ND	1.7	ND	5.5	ND	ND	1.4	

[a] Each sample was collected in the same way in Table I and assayed for EDB in the same days for each lot. [b] Mango was sliced to remove the seeds. Papaya was cut into wedge-shaped tetrasected portions, diagonal portions were collected, and the seeds were removed. Grapefruit was peeled. First they were subjected to pretreatment B and then weighed, and the remainder was subjected to pretreatment A. [c] Mango, papaya, and grapefruit were imported from the same countries in Table I. [d] ND < 0.1 μg of residue/kg of sample. [e] Results of paired t-test indicate significant differences (*, $P < 0.001$; **, $P < 0.01$) between pretreatment A and pretreatment B, and there were also close relationships between the results by them ($y = 0.18 + 1.13x$, $\gamma = 0.987$ for mango or $y = -0.22 + 1.11x$, $\gamma = 0.989$ for papaya).

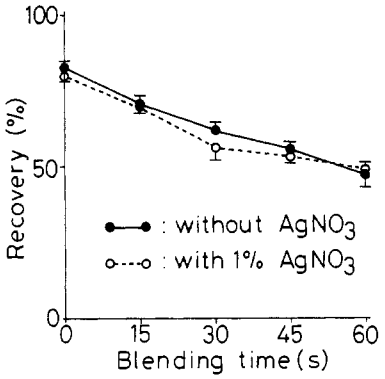

Figure 1. Effect of blending time and silver nitrate on recovery of EDB in grapefruit. After removal of the peel, blended grapefruit samples (each 150 g) were fortified with 3 μg of EDB in acetone with or without 1% $AgNO_3$. After blending during the indicated time, 100 g of each sample was weighed and assayed for EDB. The values are expressed as the means ± SD of four determinations.

differences of EDB contents in grapefruit could come from vaporization of EDB in blending or from the reaction of EDB with sulfur-containing compounds in grapefruit. Because it is well-known that some of halogenated fungicides, i.e., captan (Lukens and Sisler, 1958; Hiramatsu and Hurutani, 1977), captafol (Nutahara and Yamamoto, 1978), or dichlofluanid (Takase and Aizawa, 1981; Suzuki et al., 1987), react with SH-containing components in fruits or vegetables during their blending, to prevent reduced recovery, the addition of silver nitrate, selection of extraction solvent, or pH adjustment may be required. Figure 1 shows the effect of blending time and addition of silver nitrate on recovery of EDB from grapefruit. Lightly blended grapefruit (blended for 10 s) were fortified with 20 μg of EDB/kg of sample and 1% silver nitrate if necessary, and then their mixtures were blended for the indicated times. As shown in Figure 1, the recovery of EDB from grapefruit decreased in the course of blending time, and 30-s blending time, which had been used in the conventional method, brought about 15% decrease in recovery. Addition of silver nitrate to grapefruit gave no effect on improvement of recovery. However, these results could not give full explanation for ND values of grapefruit in Table I.

No one knows true levels of fumigant in the products. So if there are two sample pretreatments and their following analytical methods are the same, the sample pretreatment giving higher levels must be superior to another. Table II shows the analytical result for EDB in mango, papaya, and grapefruit given by two different sample pretreatments, i.e., pretreatment A and pretreatment B. As for mango and papaya, according to the regression analyses and the paired t-tests, good correlations were observed between the analytical results by pretreatment A and those by pretreatment B, and the latter showed higher levels than the former by 13 and 11% on the average in mango and papaya, respectively (Table II, footnotes). These values exhibited good correspondence with about 15%, which was shown for 30-s blending in Figure 1. Therefore, it is plausible that the residue levels obtained by pretreatment B show closer levels to true values. On the other hand, a constant relationship between levels in grapefruit was not observed, contrary to our expectations, and even ND levels of EDB were obtained in some samples by pretreatment A in spite of its existence in pretreatment B, and vice versa. These discrepancies, coupled with the results in Table I, caused concern that EDB in grapefruit cannot be accurately determined by this method.

Table III. Variation of Analytical Results of EDB Residues among the Tetrasected Grapefruit Samples[a]

	μg of residues/kg of sample[b]			
sample no.	A	B	C	D
1	ND[c]	2.2	5.0	4.1
2	3.1	ND	2.9	0.6
3	1.7	2.4	3.4	1.7
4	ND	1.7	ND	ND
5	ND	ND	3.5	ND

[a] Collected at the market. [b] After peeling they were tetrasected equally to A–D and then subjected to pretreatment B. [c] ND < 0.1 μg of residue/kg of sample.

Table III clearly shows that it is impossible to accurately analyze EDB in grapefruit by this method. After removal of the peel, individual grapefruit were tetrasected equally to A–D, which were subjected to pretreatment B separately, and their EDB residue levels were assayed. The results for each of the five fruits assayed are included in Table III to demonstrate the fluctuations in residue levels. The residue levels in A–D did not show any agreement with each other as expected, and this led us to further investigate the distribution of EDB in grapefruit.

The distribution of EDB in grapefruit has been already reported by King et al. (1980). However, they assayed only the peel and pulp without paying any attention to seeds. Table IV shows the distribution of EDB residue levels

Table IV. Distribution of EDB Residues among Pulp, Seed, and Peel of Grapefruit[a]

sample no.	μg of residue/kg of sample		
	pulp[b]	seed[c]	peel[b]
1	ND[d]	352	*e*
2	ND	290	0.13[f]
3	ND	399	ND
4	ND	349	0.35
5	ND	561	0.31
6	ND	822	0.46
7	ND	275	0.25
8	ND	574	0.08
9	ND	385	0.57
10	ND	671	0.16
11	ND	417	0.18
12	ND	147	0.38
13	ND	173	0.29
14	ND	704	0.87
15	0.51	947	3.77
16	0.36	1750	3.97
17	0.36	2080	2.86
18	0.25	2550	3.41
19	ND	1120	0.43
20	ND	1120	0.59
21	0.02	101	ND
22	0.02	122	ND
23	ND	37	ND
24	ND	ND	ND
25	0.07	112	ND
26	ND	104	ND
27	ND	147	0.86
28	0.11	130	0.22
29	ND	74	ND
30	0.07	156	0.15

[a] Samples 1–20 were imported from Florida and sampled at the market, and samples 21–30 were imported from Israel and collected at the port immediately after the routine quarantine inspection. After peeling and removal of the seeds, peel and pulp were subjected to pretreatment B. [b] 10% DC-200 was used. [c] 20% Carbowax 20M was used. [d] ND < 0.02 μg of residue/kg of pulp or peel, <0.1 μg of residue/kg of seed. [e] Not determined. [f] These residue levels in pulp and peel were corrected by subtracting the background levels of those of sample 24.

among pulp, seed, and peel of grapefruit. After removal of the peel, their seeds were removed completely from pulp with the greatest possible care and then peel and pulp were treated according to pretreatment B. Carbowax 20M, a liquid phase previously used routinely for the assay of EDB, results in a detection limit of only 0.1 μg/kg for EDB residues in grapefruit pulp. This is not sensitive enough for pulp and peel in this case so this phase was used for seeds only. Another liquid phase, DC-200, was adopted for the assays of pulp and peel because a lower detection limit, i.e. 0.02 μg/kg, could be achieved. Even with this phase a small peak equivalent to about 0.02 μg/kg of EDB residues was observed at the retention time of EDB in the gas chromatograms of pulp and peel in a sample (no. 24) in which EDB was not detected in the seeds. Therefore, the background levels of pulp and peel of sample no. 24 were subtracted from those of other samples. Results show that the maximum residue levels of EDB were 0.51 μg (pulp) (no. 15), 2550 μg (seed) (no. 18), and 3.97 μg (peel) (no. 16) of EDB residues per kilogram of sample and that only 0.25 μg of EDB residue/kg of sample was found in pulp even in the sample no. 18, which contained the maximum of 2550 μg of EDB residue/kg of seeds. If we assume the practical lower limits of detection for EDB as 1 μg/kg of sample, it will be concluded that all of the residue in grapefruit is present only in seed and peel, and not in pulp (edible portion) in the true sense of the term.

If the EDB residue level in the seed of sample no. 18 is converted to the value of pulp-containing seeds, 10.2 μg of EDB residue/kg of a mixture of pulp and seed will be obtained (calculated on the basis of the pulp weight), and this value agrees well with the results of the analytical value of grapefruit 14 days after fumigation, approximately 10 μg of EDB/kg of sample, obtained in the similated shipment to Japan at 13 °C by King et al. (1980). Considered from their results that no remarkable changes in EDB levels were observed in the period 14–35 days after fumigation, it seems that all of EDB residues are present only in seeds, not in pulp already at the 14th day after fumigation.

However, further studies are necessary to elucidate why EDB is present only in seed and not in pulp, exact time when EDB in pulp disappears, and whether EDB residues in pulp moves to seeds followed by disappearance in pulp or not.

Registry No. EDB, 106-93-4.

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