

Decrease in Organic Solvent Extractable Ethion by Grapefruit Pectin during Processing

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The effect of food components on pesticide residue was examined using citrus fruit often heated to make marmalade. Commercial grapefruit was peeled, and the parts were fractionated by extraction with several kinds of solvents (*n*-hexane, acetone, methanol, and water). The amount of organic solvent extractable ethion remaining after heating was smallest in the crude peel water extract. Purification of the water extract by deposition with methanol and dialysis produced a gel. The gel reduced the amount of several organophosphorus pesticides extracted by organic solvent during heating. From the IR spectra, the chief ingredient of the gel was considered to be pectin, which is contained in most plants. Thus, the pectin of grapefruit was considered to have masked organophosphorus pesticides from organic solvent extraction. Pectin is easily transuded from foodstuffs by cooking and appears to play a role in reducing the amount of pesticide extracted by organic solvents with heating.

Keywords: Pesticide; food; component; effect; cooking

INTRODUCTION

Pesticide residues in agricultural products are of growing concern (Handley et al., 1988; Nagayama et al., 1994, 1995; Neidert et al., 1996). The amount of pesticide residue is decreased by cooking or processing of the crop (Hori et al., 1992; Tsumura-Hasegawa et al., 1992; Nagayama et al., 1992; Nakamura et al., 1993); the effect of cooking by boiling, frizzling, or frying was found to differ with the pesticide species (Nagayama, 1996). The pesticide remaining in food is suggested to be not only directly decomposed by heating but also affected by the food components themselves, combining with or translocating into certain components during the cooking process. Accordingly, I examined the effect of food components on the decrease in the spiked amount of certain detectable pesticides after cooking in grapefruit often used to make jam or marmalade.

Herein, I report the effect of the pectin of grapefruit on the amount of organic solvent extractable ethion, a pesticide frequently detected in this fruit, and other organophosphorus pesticides.

MATERIALS AND METHODS

1. Sample and Reagents. Pesticides examined included ethion, chlorpyrifos, prothiofos, isoxathion, cyanofenphos, EPN, and dialifos.

Sample. Commercial grapefruit was obtained at a local market in Tokyo. No ethion was detected in the grapefruit purchased.

Analytical Standard and Reagents. Ethion, chlorpyrifos, prothiofos, isoxathion, cyanofenphos, EPN, and dialifos for the analytical standard were provided by Wako Pure Chemical Industries, Ltd., Osaka, Japan. Organic solvents of analytical grade were provided by Kanto Chemical Co., Inc., Tokyo, Japan, or Wako Pure Chemical Industries. Water was obtained by treatment of distilled water with Milli-Q (Millipore Corp., Milford, MA).

Lemon Pectin. Commercial lemon pectin was provided by Wako Pure Chemical Industries.

Dialysis Membrane. Seamless cellulose tubing, 27/32 type, was provided by Viskase Sales Co., Chicago, IL.

2. Processing of the Grapefruit. *Extraction of the Grapefruit Components.* Grapefruit was peeled and separated into pericarp and pulp. Samples were homogenized with *n*-hexane, acetone, methanol, and water, in that order (Chart 1). The *n*-hexane, acetone, and methanol extracts were dissolved in extracted solvent of about 5 times the concentration, respectively, while the water extract was emulsified in water at about double the volume of the sample, after concentration under reduced pressure.

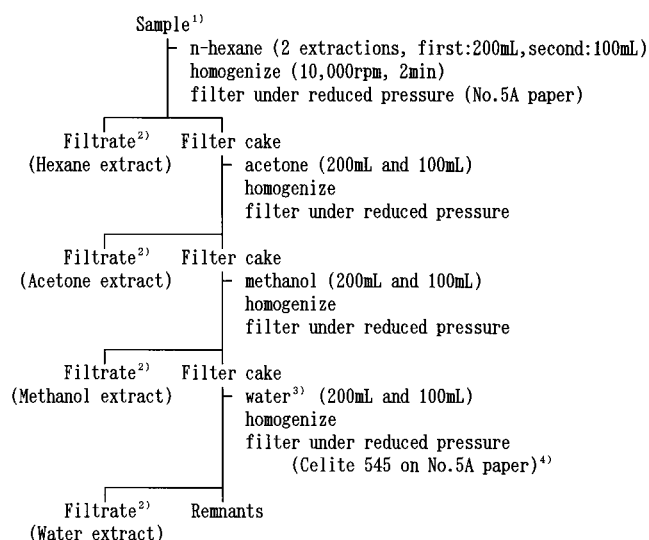
After the water extract of the peel was separated into precipitate and supernatant by methanol addition, the precipitate was dialyzed with water in a refrigerator at below 5 °C (Chart 2). All of the concentrated extracts were kept in the refrigerator until heat treatment.

Heat Treatment of Ethion and Grapefruit Extracts. Ten parts per million of ethion/*n*-hexane solution (0.1 mL) was distributed into a 10 mL centrifuge tube with a screw cap, and the solvent was vaporized by purging with air. A definite amount (0.5 g) of fresh grapefruit extracts was added to the tube and diluted to 1 mL with water after evaporation of the organic solvent used to extract. Ethion was dissolved well in the aqueous extract solution in an ultrasonic bath. After heating on an aluminum block heater at 120 °C for 20 min, the solution was left to cool.

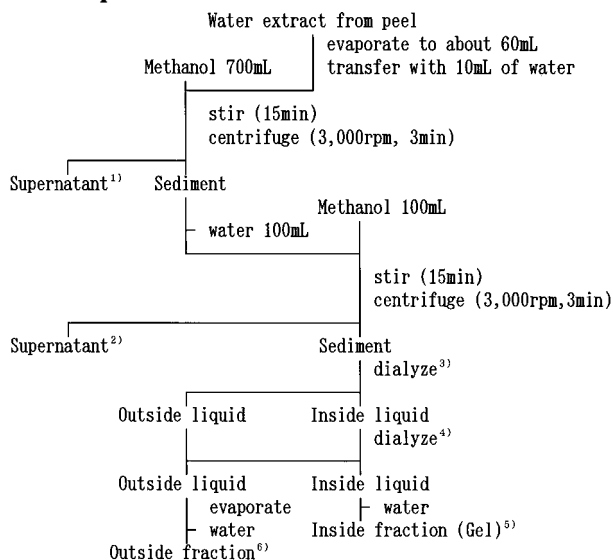
3. Analytical Method. The sample solutions were extracted with 1.0 mL of ethyl acetate after addition of sodium chloride (about 0.2 g) by shaking vigorously for 1 min. The ethyl acetate layers were separated by centrifugation (3000 rpm for 3 min), and an aliquot of 5 µL of the ethyl acetate solution was directly subjected to gas chromatography (Chart 3).

Gas chromatography was performed on a Shimadzu Model GC-14A (Shimadzu, Ltd., Kyoto, Japan) with a flame photometric detector (FPD) interfaced to a Shimadzu Chromatopac C-R4A. The gas chromatograph conditions were as follows: column, 1.5 m × 3.2 mm i.d. glass tubing packed with 2% silicone DCQF-1 on 80–100 mesh Chromosorb G AW-DMCS; column oven temperature, 200 °C; injector and detector temperature, 250 °C; carrier gas delivery rate, nitrogen at 50 mL/min, hydrogen at 60 mL/min, air at 60 mL/min. The amount of ethion was quantitated by a data processor. Under these conditions, ethion was detected at 8.2 min, and the linear calibration curve for ethion at 0.5–25.0 ng was obtained with a 5 µL injection.

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Chart 1. Fractionation of Grapefruit Components by Extraction with Solvent^a

^a (1) Grapefruit peel or pulp was used (50–150 g of grapefruit was used for one fractionation). (2) The filtrate was adjusted to 0.5–5.0 g/mL, for original material, with each extract solvent. (3) Purified with a Milli-Q system using distilled water. (4) Filtered through a 5 mm layer of Celite 545 (Wako Pure Chemical Industries) on No. 5A paper (diameter, 95 mm; Kiriya Glass Works Co., Tokyo, Japan) under reduced pressure.

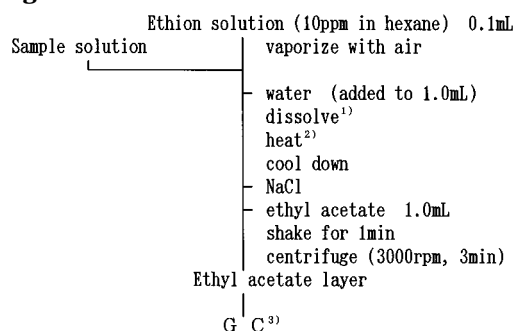
Chart 2. Fractionation of the Extract with Water from Grapefruit Peel^a

^a (1) Dissolved in methanol/water (10:1) fraction. The supernatant was adjusted to 5 g/mL for original material. (2) Dissolved in methanol/water (1:1) fraction. The supernatant was adjusted to 5 g/mL for original material. (3) Using a dialysis membrane (seamless cellulose tubing; fractionation molecular weight, 12 000–14 000) and 500 mL mess cylinder, with 300 mL of water. Separated into liquid inside and outside by stirring for 16 h at 5 °C. (4) Dialyzed twice. Separated with the dialysis membrane by stirring for 36 h at 5 °C. (5) The fraction was adjusted to 0.5 g/mL for original material. (6) The fraction was adjusted to 5 g/mL for original material.

The percentage of ethion extracted from the sodium chloride saturated solution with ethyl acetate was $99.3 \pm 5.6\%$ ($n = 10$, average \pm CV).

RESULTS AND DISCUSSION

1. Behavior of Ethion after Heating with Grapefruit Extract. The amount of ethion heated with

Chart 3. Investigation Procedure for Effect of Grapefruit Component on Ethion Residue after Heating^a

^a (1) Vibrate for 30 s using an ultrasonic bath. (2) Heat on an aluminum heater block at 120 °C for 20 min. (3) GC conditions: column, 2% DCQF-1 (Chromosorb G AW-DMCS) 80/100 mesh, 3.0 mm i.d. \times 1.5 m; detector, FPD; column temperature, 200 °C; injector and detector temperature, 250 °C; carrier gas flow rate, N₂ at 50 mL/min; injection volume, 5 μ L.

Table 1. Effect of Heating^a with Grapefruit Extract on the Amount of Organic Solvent Extractable Ethion

part	extract solvent	ethion ^b (%)	
		range	av
pericarp	<i>n</i> -hexane	94–101	98
	acetone	94–101	98
	methanol	95–98	96
	water	33–43	38
pulp	<i>n</i> -hexane	98–99	99
	acetone	99–101	100
	methanol	93–96	95
	water	92–100	97

^a In an aluminum bath at 120 °C for 20 min. ^b Extraction of ethion with ethyl acetate as a percentage of control, the amount of ethion directly extracted from aqueous solution. The values (percent) were obtained from two to four trials.

grapefruit extract was determined from residue in commercial samples. The detected level of ethion in grapefruit peel was in the range 0.07–2.0 ppm (Nagayama et al., 1995). Therefore, the equivalent of 2.0 ppm of ethion was added to each extract sample.

n-Hexane, acetone, and methanol extracted solution (0.1 mL) and water extracted solution (1 mL) (each solution containing the same amount of extract ingredient as in fresh grapefruit) were added to centrifuge tubes containing 1 μ g of ethion and heated with the screw cap tightened. One milliliter of water was similarly heated for the control sample. Using the amount of ethion extracted with ethyl acetate from the sample saturated with sodium chloride, I examined the interaction of food components and ethion. It was considered that the residual pesticide in foodstuffs was decomposed or vaporized by heating and was either immediately decomposed or combined by interactions with certain components of the foodstuffs. The amount of extractable pesticide was suggested to be reduced. Therefore, the rate of extraction of ethion from heated samples was used as an indicator of interaction, with a low rate inferring a strong effect in contrast with water alone.

Table 1 shows the levels of extractable ethion in the mixture after heating. Values are the average of more than two trials. The amount of extractable ethion in the water extract of grapefruit pericarp after heating was one-third of that before heating. More than 95% was extracted after heating with other extracts.

Next, the water extract of grapefruit peel was further

Table 2. Effect of Heating with Water Extract of Grapefruit on the Amount of Organic Solvent Extractable Ethion

fraction	ethion ^a (%)	
	range	av
10:1 ^b dissolved	97–105	102
1:1 dissolved	93–97	94
sediment	42–65	54

^a Level of ethion extracted with ethyl acetate as the control; the amount of ethion directly extracted from aqueous solution. The values (percent) were obtained from two to three trials. ^b Ratio of methanol and water.

Table 3. Effect of Heating with Dialyzed Liquid of Grapefruit on the Amount of Organic Solvent Extractable Ethion

fraction	ethion ^a (%)	
	range	av
inner liquid		
1:1 ^b dissolved	82–89	85
water dissolved	97–105	101
water insoluble gel	74–84	78
outside liquid		
1:1 dissolved	92–102	98
1:1 sediment	91–103	95

^a Level of ethion extracted with ethyl acetate as the control; the amount of ethion directly extracted from aqueous solution. The values (percent) were obtained from three trials. ^b Ratio of methanol and water.

fractionated using methanol. The extract was then added to 10 times the volume of methanol, drop by drop, and stirred for 30 min. Next, it was centrifuged for 10 min at 3000 rpm and separated into supernatant and sediment. The treatment was repeated, and the sediment was again dissolved in water, to which an equivalent volume of methanol was added, drop by drop, and stirred for 30 min twice. The crude water extract of grapefruit peel was separated into three fractions, methanol/water (10:1) dissolved, methanol/water (1:1) dissolved, and sediment. Ethion (1 μ g) was heated with each of these three fractions in a centrifuge tube. The amount of extractable ethion in sediment was 35% after heating, while that in the other fractions was <7% (Table 2).

The sediment fraction of the grapefruit water extract in methanol/water (1:1) was subsequently separated using a dialysis membrane into high (inside) and low (outside) molecular weight fractions, above and below 12 000–14 000, respectively. These fractions were treated with methanol again. The inside fraction sedimented in methanol/water (1:1) was well washed with water and separated into water soluble and insoluble fractions. The insoluble fraction formed a gel. The percentage of extractable ethion, remaining in each fraction after heating, is shown in Table 3. The greatest decrease in ethyl acetate extractable ethion was seen in the water insoluble gel fraction of peel extracted with water, with a molecular weight of more than 12 000–14 000.

2. Effect of Water-Extracted Gel Fraction on Ethion by Heating. *Effect of the Mass of Gel.* The above water insoluble gel fraction (0.0, 0.2, 0.5, 1.0, 2.0, and 5.0 mL) was distributed into centrifuge tubes containing 1 μ g of ethion. Each test solution was made up to 5.0 mL with water and heated. Table 4 shows the average level of extractable ethion in the tubes after heating relative to the control (directly extracted from centrifuge tubes containing 5 mL of a solution of ethion

Table 4. Effect of Heating on the Amount of Organic Solvent Extractable Ethion

liquid ^a vol (mL)	ethion ^b (%)	
	range	av
0.0		100
0.2	101–110	107
0.5	89–101	95
1.0	87–95	91
2.0	84–91	88
5.0	76–83	80

^a Heated with dialyzed inner gel from grapefruit extracted with water. The values (percent) were obtained from two trials. ^b The rate of the ethion amount extracted with ethyl acetate compared to that with only water (liquid volume 0.0 mL).

Table 5. Effect of Heating Temperature on the Amount of Organic Solvent Extractable Ethion

temp ^a (°C)	ethion ^b (%)	
	range	av
30	98–99	99
60	90–96	93
80	87–94	91
100	85–86	86
120	71–79	75

^a Heated with 2 mL of the dialyzed inner gel from grapefruit extracted with water. The values (percent) were obtained from two trials. ^b Level of ethion extracted with ethyl acetate as the control; the amount of ethion directly extracted from aqueous solution.

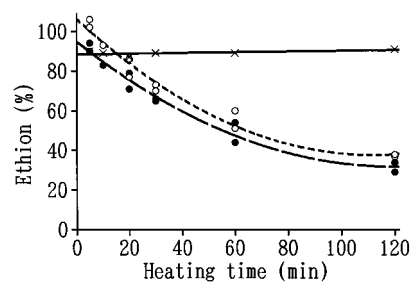


Figure 1. Extracting percentage of ethion in gel extracted from grapefruit peel after heating in an aluminum bath at 120 °C: (●) with 2 mL of the gel; (○) in 2 mL of water; (×) extracting ratio of ethion in the gel to that in water. Values for ethion (percent) are the average of two trials.

in water). The amount of extractable ethion decreased in the heated solution with increasing mass of the gel. Accordingly, the extraction ratio was considered to correlate to the mass of the gel.

Influence of Heating Temperature. The water insoluble gel fraction (2.0 mL) was distributed into centrifuge tubes containing 1 μ g of ethion and heated in an aluminum block bath at 30, 60, 80, 100, and 120 °C for 20 min. The control tubes were not heated. Table 5 shows the amount of extractable ethion in the tube after heating compared to the control. The amount extracted was considered to relate to the heating temperature.

Influence of Heating Time. The water insoluble gel fraction (2.0 mL) was distributed into centrifuge tubes containing 1 μ g of ethion and heated for 5, 10, 20, 30, 60, or 120 min. Figure 1 shows the decrease in extractable ethion during heating in the gel or in water as a control. The amount decreased after heating of the gel. The extractable amount of ethion after heating in water also decreased relative to the heating period, but the level of the decrease was less than with the gel fraction for all heating periods. The ratio of ethion extracted by heating from the gel in comparison to that from water was similar [the ratio was $89.2 \pm 1.2\%$

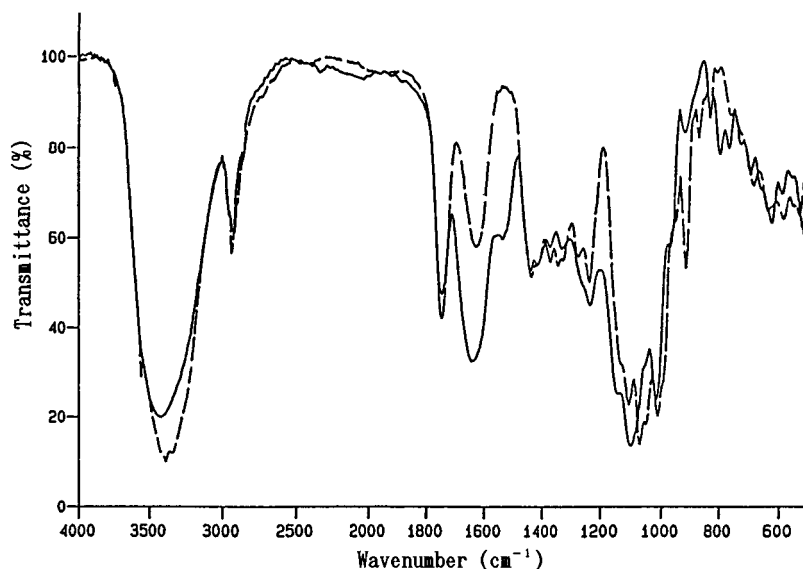


Figure 2. Comparison between the gel of grapefruit extract and commercial lemon pectin in IR spectrum: (—) gel of grapefruit extract; (---) commercial lemon pectin.

(average \pm SD)] for all periods of heating. Hence, ethion was gradually decreased with decomposition or vaporization in water by heating, and the component in the gel fraction was considered to interfere with ethion extraction with organic solvent during heating.

3. Identification of the Gel. *Comparison of IR Spectra.* The principal ingredient of the gel fraction, which had a major role in the decrease in ethion extraction during heating, was investigated. Methanol was added and the gel fraction concentrated under reduced pressure until the water completely evaporated. The dried gel was then left to stand for 2 days in a reduced pressure desiccator, and the IR spectrum was obtained with a KBr tablet after the moisture was removed. In addition, the IR spectrum of commercial lemon pectin was similarly measured for comparison. The IR spectra of each fruit were similar (Figure 2).

Influence of Pectinase Treatment. Amounts of 0.8 and 2.5 mL of 1% pectinase aqueous solution were added to 4.0 and 2.5 mL of the gel fraction, respectively. These mixtures were maintained at 40 °C for 16 h, and then 0.6 mL of the first mixture was diluted to 1.0 mL with water; 1.0 mL of the treated mixtures and the nontreated (only maintained at 40 °C for 16 h, nontreatment of pectinase) gel fraction were distributed in centrifuge tubes containing 1 μ g of ethion and heated in an aluminum block bath at 120 °C for 20 min. The commercial lemon pectin (the concentration was 0.35%, same as the gel from grapefruit) was also tested. The amount of extractable ethion decreased to 79% in the gel fraction, but only to 97–99% in the pectinase-treated gel fraction and the pectinase solution, compared with the pectinase solution only. The amount of extractable ethion decreased to 93% in the commercial pectin solution but only to 102% in the pectinase-treated commercial pectin solution, compared with the pectinase solution only. The decrease in the pectinase-treated fraction after heating was similar to that in the control with the pectinase solution only but differed from that in the nontreated gel (Figure 3).

From the IR spectra, the chief ingredient of the gel was considered to be pectin, which is contained in most plants. From the slight decrease in extractable ethion after heating by pectinase treatment, it was concluded that pectin is involved in reducing the amount of ethion

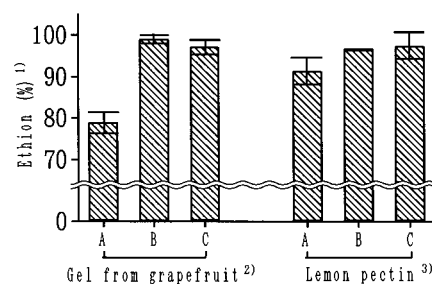


Figure 3. Effect of pectinase treatment of gel from grapefruit and commercial lemon pectin on the amount of extractable ethion after heating: (1) ratio of the amount of extractable ethion to the amount of ethion extracted from 1% pectinase solution [values for ethion (percent) are the average of two trials]; (2) 0.35% gel extracted from grapefruit; (3) 0.35% solution of lemon pectin on the market; A, nontreatment solution; B, after treatment with one-fifth the volume of 1% pectinase solution in the gel; C, after treatment with an equivalent volume of 1% pectinase solution in the gel.

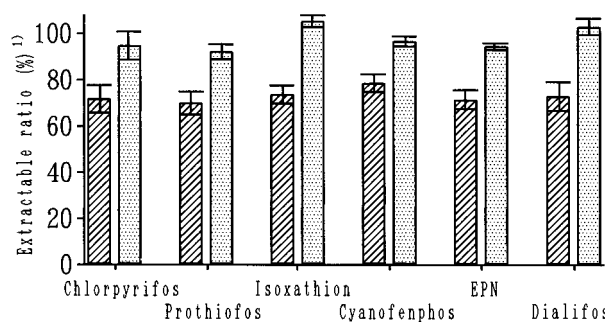


Figure 4. Effect of pectinase treatment of gel from grapefruit on the amount of extractable organophosphorus pesticide after heating: (1) extraction of ethion with ethyl acetate as a percentage of that of 1% pectinase solution. (values are the average of two trials); (slashed bar) gel from grapefruit; (dotted bar) pectinase-treated gel from grapefruit.

extracted during the heating process. The influence of heating on several other organophosphorus pesticides was found to be the same as that on ethion (Figure 4): Therefore, pectin likely reduces the amount of organophosphorus pesticides extracted during heating.

4. Conclusions. The water insoluble gel fraction of grapefruit peel was shown to cause a reduction in the amount of organic solvent extractable pesticide in grapefruit products during heating. The chief ingredi-

ent of the gel was considered to be pectin. Pectin, a common component of most plants, is utilized to make marmalade from citrus fruit peel. The pesticides remaining in the foodstuffs were decomposed and vaporized during heating, and the amount of pesticide extractable with organic solvent was suggested to be reduced by interaction with pectin. Pectin transuded by heating is considered to play a major role in reducing the amount of pesticide extracted from foodstuff.

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