

Behavior of Residual Organophosphorus Pesticides in Foodstuffs during Leaching or Cooking

Toshihiro Nagayama*

The Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1 Hyakunincho, Shinjuku-ku, Tokyo 169, Japan

The relationship between cooking procedure and pesticide species on pesticide residues in raw crops was examined using pesticide-containing commercial green tea leaves, spinach, strawberries, oranges, and grapefruit. The residual organophosphorus pesticides in green tea leaves and in crops were decreased on leaching or cooking, and the rate of decrease was closely related to the octanol–water partition coefficient. The regression expression $\log(L_r) = 2.25 - 0.312 \log K_{ow}$, $r = 0.923$, where L_r is the translocated pesticide concentration in drawn tea (%) and K_{ow} is the partition coefficient (octanol/water) of the pesticide, was shown from the relationship between the amount of organophosphorus pesticides in the tea leaves and the amount dissolved into the brewed tea. On the other hand, the decrease of organophosphorus pesticide residues in spinach after 5 min of boiling or in an orange after cooking and the pesticide K_{ow} fit the equation $\log(P_r) = 1.59 + 0.06 \log K_{ow}$, $r = 0.991$, in boiled spinach, $\log(P_r) = 0.51 + 0.40 \log K_{ow}$, $r = 0.978$, in orange marmalade, and $\log(P_r) = -5.28 + 2.12 \log K_{ow}$, $r = 0.946$, in orange candied peel, where P_r is the percent of pesticide remaining in the fresh state. The pesticide remained in the processed food according to the K_{ow} expression. The relationship was similar for the pesticide residues after repeated cooking by boiling, frizzling, or frying.

Keywords: Regression; pesticide; leaching; foodstuff; cooking

INTRODUCTION

Since most crops are eaten after cooking, the pesticides used on the crops are vaporized and decomposed during the cooking or processing of the crop before ingestion.

Depending on the distribution of the pesticides in the crop, that is, adhered to the outside of the crop, combined with some materials of the crop or translocated into the crop tissue, the dissipation rate of the pesticides from the crop may not be the same. The practical correlation of pesticide loss is accordingly obtained by examining the sample on the market.

Several studies have been made on the dissipation of pesticides in crops after cooking (Archer, 1975; Hearnberger et al., 1976; Ei-Zemaity, 1988; Hori et al., 1992; Tsumura-Hasegawa et al., 1992; Nakamura et al., 1993). However, the dissipation has been examined using an experimentally treated sample, and the general rules for the behavior of the pesticide have not been established.

Herein, we will report the rules for the dissipation rate of pesticides in marketed crops during leaching or cooking.

MATERIALS AND METHODS

Materials. 1. *Samples. Green Tea Leaves.* Six brands of green tea leaves (Sencha) were obtained at a department store in Tokyo. The residual amounts of the organophosphorus pesticides EPN, (*E*)-chlorfenvinphos (CVP-E), CVP-Z, fenitrothion (MEP), isoxathion, methidathion (DMTP), and prothiophos in the tea leaves were measured. The residual concentrations of EPN, CVP-E, CVP-Z, MEP, isoxathion,

DMTP, and prothiophos were 0.41, 1.1, 2.0, 0.05, 2.8, 0.02, and 1.5 ppm, respectively.

Spinach. Commercial spinach was obtained at a local market in Tokyo. They contained 0.33, 0.07, 2.5, 11, and 0.91 ppm EPN, cyanofenphos (CYP), dialifos, isoxathion, and prothiophos, respectively.

Strawberries. Commercial strawberries were obtained at a local market in Tokyo. They contained 0.28–0.42 and 0.28–0.52 ppm dialifos and prothiophos, respectively.

Oranges. Commercial oranges were obtained at a local market in Tokyo. They contained 0.28–0.58, 0.38–0.69, 0.21–0.44, and 1.1–2.5 ppm MEP, mecarbam, DMTP, and piri-daphenthion, respectively.

Grapefruit. Commercial grapefruit was obtained at a local market in Tokyo. The concentration of ethion was 0.22–1.4 ppm.

Soybean Oil. Soybean oil was provided by Kanto Chemical Co., Inc., Tokyo, Japan. EPN, CYP, dialifos, isoxathion, and prothiophos were not contained in this oil.

Analytical Standards and Reagents. All kinds of pesticides for the analytical standards were provided by Wako Pure Chemical Industries, Ltd., Osaka, Japan. Organic solvents were of pesticide analysis grade.

2. Processing of the Crop. *Brewing Tea.* The tea leaves (5 g) were immersed in 300 mL of boiling water for 5 min. Then, they were again immersed in boiling water (second brew). The tea leaves after brewing, the brewed tea, and the second brew were analyzed separately.

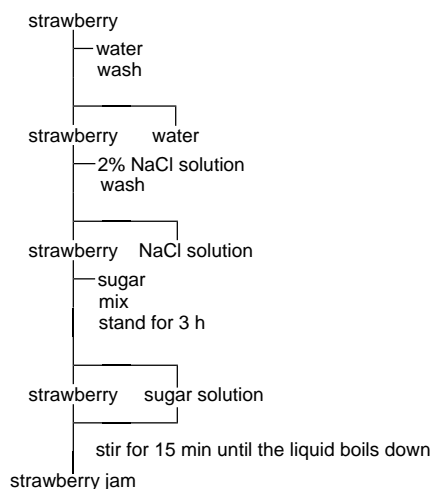
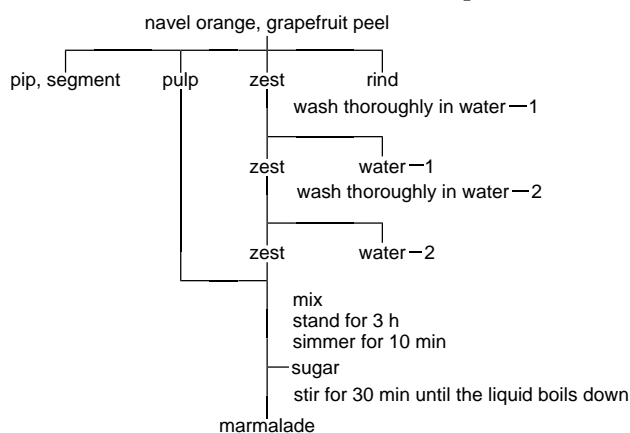
Spinach Boiling. Spinach (10 g) was immersed in 100 mL of boiling water, and heated for 2, 5, and 10 min.

Spinach Frizzling. Spinach (10 g) was frizzled with 0.5 mL of soybean oil for 2 or 5 min.

Jam. Decalaxed strawberries (70 g) were rinsed with 200 mL of distilled water and 400 mL of 2% sodium chloride solution. The rinsed strawberries and sugar (equivalent to 40% of the strawberries) were blended and reduced until the liquid boiled down and turned into paste (Chart 1).

Marmalade. Oranges and grapefruit (each 400 g) were peeled and separated into zest, rind, juice sac, segment, and pip. After the zest was washed with 1000 mL of distilled water twice, the washed zest and juice sac were blended and

* Present address: The Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1 Hyakunincho, Shinjuku-ku, Tokyo 169, Japan.

Chart 1. Procedure of Strawberry Jam Preparation**Chart 2. Procedure of Marmalade Preparation**

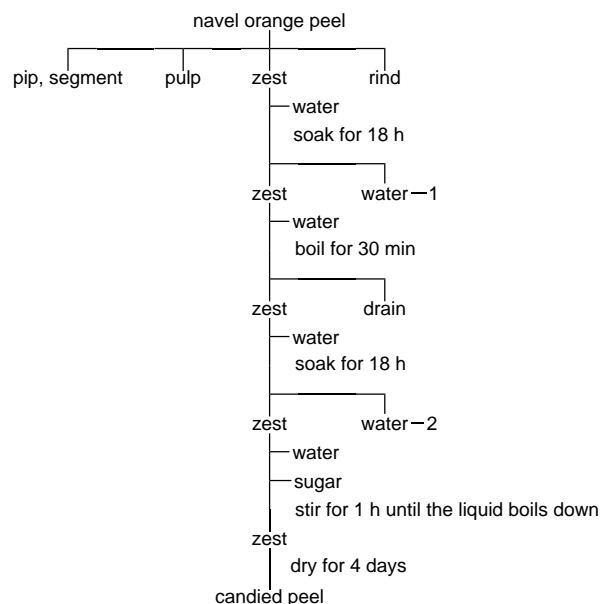
simmered for 10 min. Sugar was added (equivalent to the zest and juice sac mix) and stewed for 30 min into paste (Chart 2).

Candied Peel. An orange (200 g) was peeled and separated into zest, rind, juice sac, segment, and pip. After soaking in 400 mL of distilled water for 18 h, it was boiled in another 400 mL of distilled water for 30 min. The water was drained. It was soaked in another 350 mL of distilled water for 18 h. The zest was reduced with 300 mL of distilled water and sugar (150 g) for 1 h; the liquid was boiled down and dried for 4 days at room temperature (Chart 3).

Analytical Method. The Japanese official method for regulative organophosphorus pesticides (The Livelihood Hygiene Bureau of the Ministry of Public Welfare, Japan ed., 1993; this method was amended in March 1996) was slightly improved; the sample volume was decreased from 150 to 20 g, and the reextracted solution was changed from benzene to dichloromethane and used for analysis of pesticide residues. The recovery of organophosphorus pesticide to various samples was greater than 80%, at the 0.1 ppm spiking levels.

For analysis of organophosphorus pesticides in crops and dishes, each solid moist sample (20 g) was extracted with 200 mL of acetone. The dry tea leaves stood for 30 min with 3-fold water and was then extracted with acetone. Immediately after addition of 5% NaCl, the brewed tea and the cooking water were extracted with dichloromethane.

The extracts were evaporated under reduced pressure below 40 °C. The residue was diluted with 100 mL of 5% NaCl solution, and the mixture was extracted with 100 and 50 mL of dichloromethane twice. The dichloromethane layers were collected, dehydrated with anhydrous sodium sulfate, and evaporated to dryness under reduced pressure below 40 °C. Except for a nonoil sample (5 g of soybean oil and the extracts from frizzling spinach and citrus peel), the residue was dissolved in 25 mL of *n*-hexane and extracted twice with 30 mL of acetonitrile saturated with *n*-hexane. The acetonitrile

Chart 3. Procedure of Candied Peel Preparation

layers were collected and washed with 50 mL of *n*-hexane saturated with acetonitrile. The acetonitrile layer was evaporated to dryness under reduced pressure below 40 °C, and the small amount of solvent remaining was vaporized by purging with nitrogen gas. The residue was dissolved in ~1–2 mL of *n*-hexane.

The tea leaves and spinach samples were refined by column chromatography. The acetone extract was transferred to a column packed with a mixture of cellulose and charcoal (for column chromatography, 10 + 1). After elution with 200 mL of acetone, the eluate was evaporated to dryness under reduced pressure below 40 °C and a small amount of the solvent was vaporized with nitrogen gas. The residue was dissolved in ~1–2 mL of *n*-hexane.

An aliquot of 5 µL of the solution was directly subjected to gas chromatography.

Gas chromatography was performed on a Shimadzu Model GC-9A (Shimadzu, Ltd., Kyoto, Japan) with a flame photometric detector (FPD) and a flame thermionic detector (FTD) interfaced to a Shimadzu Chromatopac C-R2AX. The following gas chromatograph conditions were used: a column of 1.5-m × 3.2-mm i.d. glass tubing packed with 2% Silicone DCQF-1 on 80–100-mesh Chromosorb G AW-DMCS for FPD and 1.0-m × 3.2-mm i.d. glass tubing packed with 3% Silicone OV-101 on Chromosorb W AW-DMCS for FTD; column oven at 180–250 °C depending on the pesticide; detector at 250 °C; injector at 250 °C; carrier gas helium at 50–60 mL/min; hydrogen at 60 (FPD) and 3.5 (FTD) mL/min; air at 60 (FPD) and 150 (FTD) mL/min. The pesticide was detected at a retention time of 5–10 min. The amount of pesticide was quantitated by a data processor.

RESULTS AND DISCUSSION

1. Organophosphorus Pesticides Leaching into Brewed Tea. Two percent to 52% of the organophosphorus pesticides in the tea leaves were translocated into the brewed tea (Nagayama et al., 1989). This leaching ratio varied with the pesticide but not with the residue concentration. It is considered to be related to water solubility. The relationship between the percentage of pesticide translocated into the brewed tea (leaching ratio) and the water solubility of the pesticide showed a regression line (Figure 1)

$$\log(L_r) = 0.43 + 0.45 \log(W_s) \quad r = 0.896$$

where L_r , the leaching ratio (%), and W_s , the water solubility of the pesticide, were obtained.

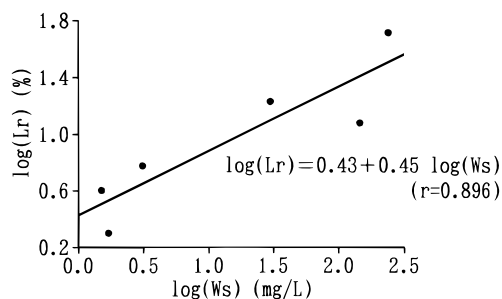


Figure 1. Regression line between water solubility and amount of pesticide in brewed tea (L_r , leaching ratio; W_s , water solubility).

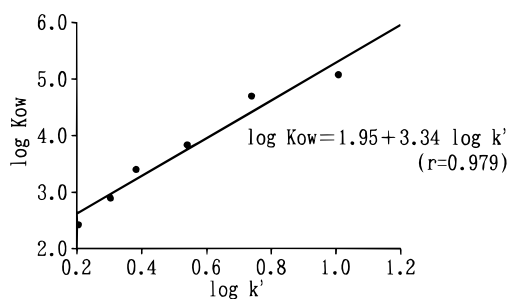


Figure 2. Regression line between HPLC capacity factors (k') and the octanol-water coefficients (K_{ow}).

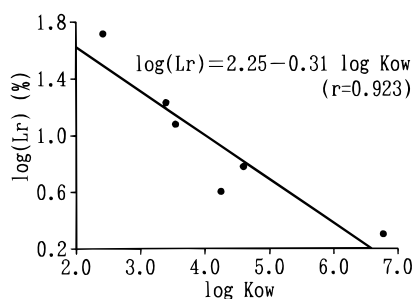


Figure 3. Regression line between K_{ow} and the amount of pesticide in brewed tea (L_r , leaching ratio).

On the other hand, a satisfactory relationship has been observed between the water solubility and the partition coefficient of octanol/water (K_{ow}) (Chiou et al., 1977). Accordingly, the leaching ratio was considered to be related to K_{ow} . However, the K_{ow} 's of the surveyed pesticides were not identified. K_{ow} was determined by high-performance liquid chromatography (HPLC), utilizing a calibration curve previously obtained by using reference standards with known K_{ow} values (McCall, 1975; Finizio et al., 1991). The retention time was determined with a reversed-phase ODS column (5- μ m particle size, 4.0-mm i.d. \times 250 mm), a solvent of methanol/water (70/30, v/v), and a flow rate 0.8 mL/min. The capacity factor (k') was calculated from

$$\log k' = \log(t_r - t_0)/t_0 \quad (1)$$

where t_0 represents the elution time of the unretained peak. $\log K_{ow}$ values were calculated from eq 2, obtained from the linear regression analysis of the reference compounds malathion, parathion, fenitrothion, dialifos, and methidathion (Figure 2):

$$\log K_{ow} = 1.95 + 3.34 \log k' \quad (n = 6, r = 0.979) \quad (2)$$

The regression line showed a $\log K_{ow}$ for EPN of 4.60, for isoxathion of 4.25, and for prothiophos of 6.77.

Figure 3 shows the relationship between K_{ow} and the leaching ratio. The regression expression

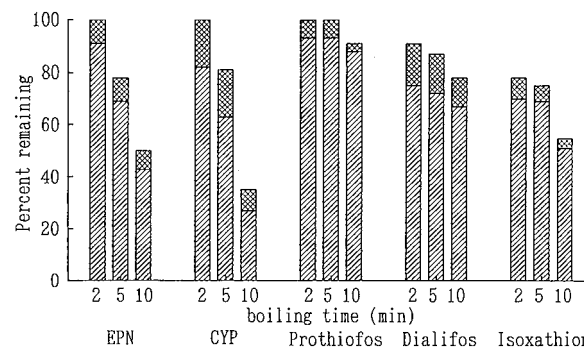


Figure 4. Effect of boiling on pesticide residues in spinach: (slashed bar) in boiled spinach; (cross-hatched bar) in cooking water.

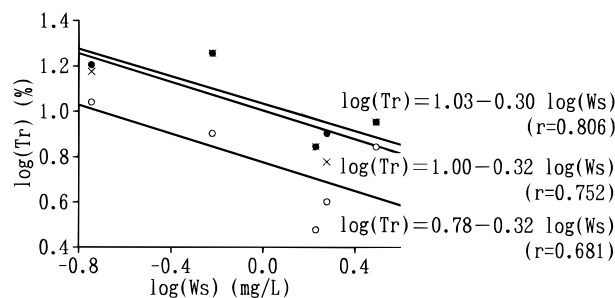


Figure 5. Relationship between water solubility and pesticide translocation into the cooking water: (●) boiled for 2 min, (×) boiled for 5 min, (○) boiled for 10 min. (T_r , translocation ratio; W_s , water solubility).

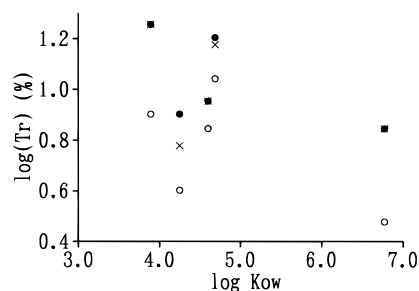


Figure 6. Relationship between K_{ow} and pesticide translocation in the cooking water: (●) boiled for 2 min, (×) boiled for 5 min, (○) boiled for 10 min (T_r , translocation ratio).

$$\log(L_r) = 2.25 - 0.31 \log K_{ow} \quad r = 0.923$$

was obtained. The pesticides with large K_{ow} values probably entered the tissue strongly, combining with it and not moving with the circulation water. These pesticides are deservedly insoluble in water during the cooking process.

2. Translocation and Residue of Pesticides in Spinach after Cooking. *Boiling Treatment.* The level of pesticide in spinach after boiling and in the cooking water decreased with boiling time (Figure 4). The residual level differed with each pesticide.

The pesticides in the tea leaves were translocated according to the water solubility or K_{ow} . Hence, the relationship between the translocation rate in the cooking water and the water solubility or K_{ow} was examined. The pesticides in spinach were translocated into the cooking water according to the water solubility, not to K_{ow} (Figures 5 and 6). The regression expressions

$$\log(T_r) = 1.03 - 0.30 \log(W_s) \quad r = 0.806$$

for 2 min of boiling,

$$\log(T_r) = 1.00 - 0.32 \log(W_s) \quad r = 0.752$$

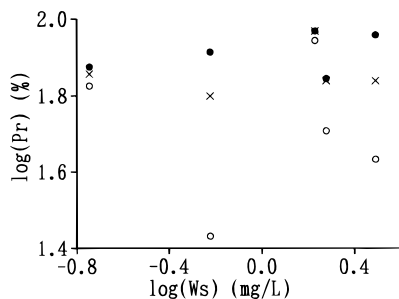


Figure 7. Relationship between water solubility and pesticide residue in boiled spinach: (●) boiled for 2 min, (×) boiled for 5 min, (○) boiled for 10 min (P_r , percent remaining; W_s , water solubility).

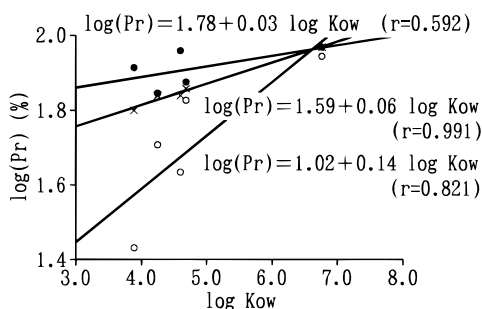


Figure 8. Relationship between K_{ow} and pesticide residue in boiled spinach: (●) boiled for 2 min, (×) boiled for 5 min, (○) boiled for 10 min (P_r , percent remaining).

for 5 min, and

$$\log(T_r) = 0.78 - 0.32 \log(W_s) \quad r = 0.681$$

for 10 min, where T_r is the percent of pesticide translocated into the cooking water from the spinach (translocation ratio) and W_s is the water solubility of the pesticide, were obtained. These inclinations and the constant were similar. The pesticide translocated into the cooking water was gradually decreased by vaporization or resolution, but the pesticide presumably remained in the cooking water according to the water solubility.

On the other hand, the amount of residual pesticide in the boiled spinach was related to K_{ow} , not to water solubility (Figures 7 and 8). After 2 min of boiling treatment, the pesticide in the spinach was not settled for water or spinach, so the coefficient of correlation was low. The regression expression

$$\log(P_r) = 1.78 + 0.03 \log K_{ow} \quad r = 0.592$$

where P_r is the residual pesticide level in the boiled spinach (%) from the amount of raw spinach, was obtained. After over 5 min of treatment, the residual pesticide in the spinach decreased in relation to K_{ow} , the pesticide residue in spinach, and K_{ow} was fitted to the equation

$$\log(P_r) = 1.59 + 0.06 \log K_{ow} \quad r = 0.991$$

for 5 min boiling and

$$\log(P_r) = 1.02 + 0.14 \log K_{ow} \quad r = 0.821$$

for 10 min. The inclination of the regression expression increased with time, suggesting that the pesticide was gradually degraded by heat. The three regression lines joined to one point at a K_{ow} value of 6.6, suggesting difficulty of the translocation of the pesticide in the spinach into the cooking water and little degradation above $6.6K_{ow}$.

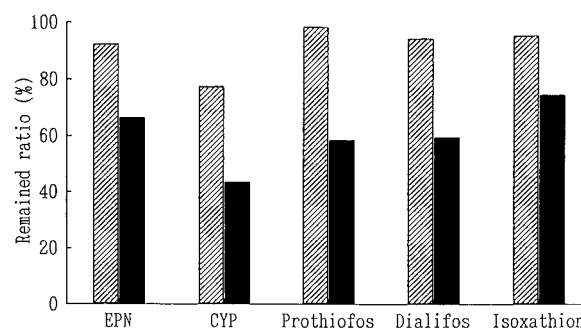


Figure 9. Effect of frizzling on pesticide residues in spinach: (slashed bar) frizzled for 2 min; (solid bar) frizzled for 5 min.

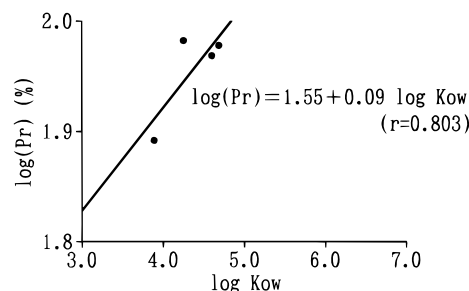


Figure 10. Regression line between K_{ow} and pesticide residue in frizzled spinach (P_r , percent remaining).

Frizzle Treatment. The frizzled spinach and the cooking oil were eaten together; therefore, both of them were examined together.

Spinach containing organophosphorus pesticides similar to the boiling experiment was used. The residual pesticide level of the frizzled spinach was decreased in proportion to treatment time with each pesticide (Figure 9). Since the spinach was charred by 5 min of frizzling, it was concluded that the pesticide directly underwent thermal degradation and was volatilized and removed. The relationship between the amount remaining in the spinach and K_{ow} was examined by the use of the 2-min frizzling data.

More than 96% prothiofos, dialifos, and isoxathion remained. The K_{ow} values for prothiofos, dialifos, and isoxathion were 6.77, 4.69, and 4.25, respectively. Since these residual levels were similar, the residue of these pesticides was probably the greatest among the pesticides in this condition. The lowest residual level was 78%, which was for cyanofenphos (CYP); the level was as high as that for boiling on the supposition of a high-temperature process. The oil used for cooking was considered to have prevented the decrease of the pesticide. Figure 10 shows the regression line between K_{ow} and the amount of pesticide remaining in the spinach frizzled for 2 min. The regression expression

$$\log(P_r) = 1.55 + 0.09 K_{ow} \quad r = 0.803$$

where P_r is the percentage of pesticide remaining in the frizzled spinach from the raw spinach, was obtained.

3. Residue of Pesticides in Strawberries after Cooking. Strawberry jam was prepared. The organophosphorus pesticides in the strawberries decreased with the preparation process (Figure 11). The pesticide in the fresh strawberries was slightly translocated into the water, with only a slight decrease during the heating process, both below 10%. Ninety-six percent and 73% of the prothiofos and dialifos in the fresh strawberries remained in the jam, respectively, and the $\log K_{ow}$ values for them were 6.77 and 4.69, respectively.

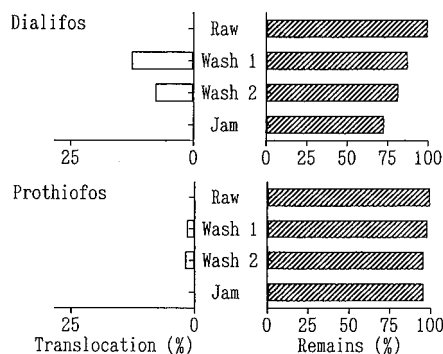


Figure 11. Behavior of organophosphorus pesticides during the preparation of strawberry jam: (slashed bars) in strawberry; (open bars) in water.

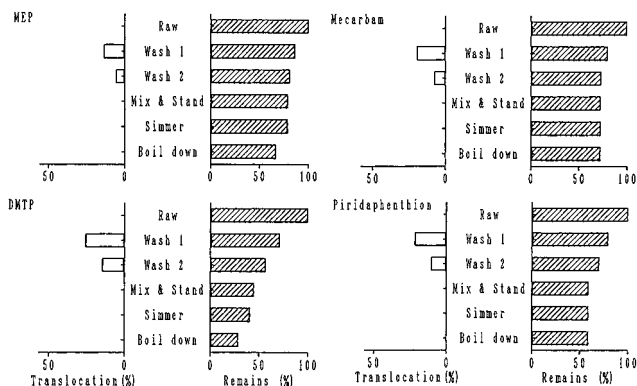


Figure 12. Behavior of organophosphorus pesticides in oranges during the preparation of marmalade: (slashed bars) in oranges; (open bars) in water.

Hence, the residual level of pesticide in the jam was related to K_{ow} , as for spinach.

4. Residue of Pesticides in Oranges and Grapefruit after Cooking. Marmalade Preparation. (1) Oranges.

The level of organophosphorus pesticides in the oranges decreased with the preparation process (Figure 12). Twenty percent to 45% of the pesticides in the fresh oranges were translocated into the water during the washing. The translocation ratio of DMTP was the highest, followed by piridaphenthion, mecarbarn, and MEP, in this order. During the heating process, the levels of MEP and DMTP were decreased by 15% and 35%, respectively, but that of mecarbarn and piridaphenthion was not reduced more than 2%. Twenty-nine percent of the DMTP in the fresh oranges remained in the marmalade, but the levels of the other pesticides in the marmalade were ~59–73% of those in the fresh oranges.

Grapefruit. Forty-five percent of the ethion in the fresh grapefruit was translocated into the water during the washing, and 15% was lost during the heating process. The residual level in the marmalade was 42% (Figure 13), being slightly different from that in orange marmalade, probably because the thickness and the constituent parts of the grapefruit zest were thin and different in comparison with orange zest.

(2) Candied Peel. The orange zest was cooked to candied peel as a confectionery material. The orange zest containing four kinds of organophosphorus pesticides was cooked, experimentally.

The level of pesticide in the orange zest decreased with the cooking process, as in the marmalade preparation process. By washing and boiling, ~15–45% of the pesticide in the fresh orange was translocated into the wash water and cooking water (Figure 14). The level

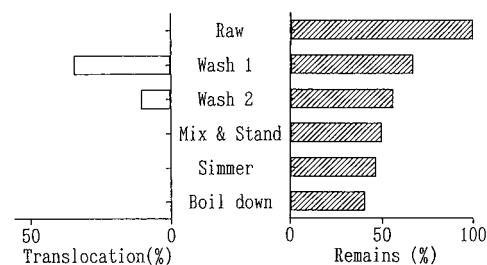


Figure 13. Behavior of ethion in grapefruit in the preparation of marmalade: (slashed bars) in grapefruit; (open bars) in water.

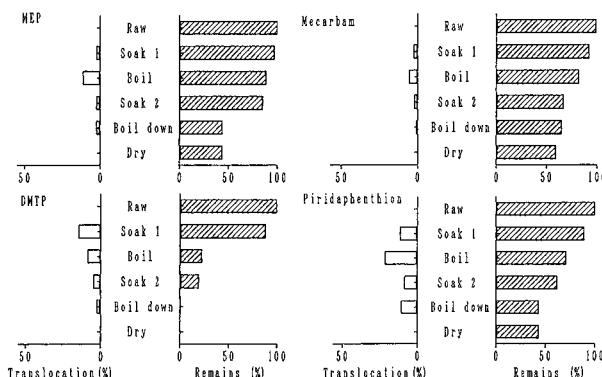


Figure 14. Behavior of organophosphorus pesticides in oranges during the preparation of candied peel: (slashed bars) in oranges; (open bars) in water.

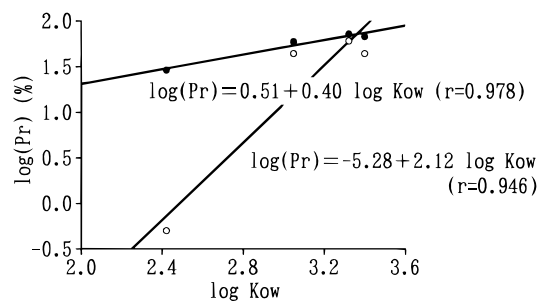


Figure 15. Relationship between K_{ow} and pesticide remaining in the cooked orange: (●) marmalade, (○) candied peel (P_r , percent remaining).

of DMTP was decreased 80% by the boiling treatment and 94% by the simmer treatment, a marked decrease compared with the other pesticides. DMTP was considered to be easily vaporized and decomposed during the heating process.

On the other hand, the level of pesticide remaining in the cooked spinach was correlated with K_{ow} . Accordingly, the residual level in marmalade and candied peel was also considered to be related to K_{ow} . The K_{ow} values of mecarbarn and piridaphenthion were determined by HPLC. The regression line gave a $\log K_{ow}$ of 3.32 for mecarbarn and 3.05 for piridaphenthion.

Figure 15 shows the relationship between K_{ow} and the residual level. The regression expressions

$$\log(P_r) = 0.51 + 0.40 \log K_{ow} \quad r = 0.978$$

in orange marmalade and

$$\log(P_r) = -5.28 + 2.12 \log K_{ow} \quad r = 0.946$$

in orange candied peel, respectively, where P_r is the percent of pesticide remaining in the orange marmalade and candied peel for the fresh orange, were obtained. K_{ow} was suggested to be related to the amount of

pesticide remaining in the processed food. The inclination of the regression expression in candied peel was steeper than in marmalade, and the amount of residual pesticide was lower in the candied peel than in the marmalade. This was attributed to the repeated heating processes and long heating time in the candied peel preparation.

Conclusions. During the cooking process, the pesticide residue in the crop decreased. Some residual pesticides were translocated into the cooking water from the raw materials according to the water solubility expression, and the pesticide remained in the processed food according to the K_{ow} expression. These relationships were shown by simple equations. The inclination of the regression expression was similar with the same cooking process and increased with cooking time. By future examination, the behavior of the pesticides remaining in the raw crops during leaching and cooking should be confirmed. These relationships were concluded to be applicable to the pesticide residue after cooking by boiling, frizzling, or frying.

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