

Pesticide Removal Efficiencies of Soybean Oil Refining Processes

Makoto Miyahara* and Yukio Saito

Division of Foods, National Institute of Hygienic Sciences, 1-18-1 Kamiyoga Setagaya-ku, Tokyo 158, Japan

Soybeans, soybean flakes, crude oil, alkali-refined oil, and bleached oil were spiked with four kinds of pesticides (dichlorvos, malathion, chlorpyrifos, and captan) and were subjected to roasting, extraction, alkali refining, bleaching, and deodorizing under laboratory conditions. The pesticide levels in the products and the byproducts were determined immediately after processing. The results of these studies have established the following: (1) The dichlorvos in the dry soybeans and the oils is decreased markedly at each stage of processing. Malathion in crude oil and alkali-refined oil is reduced at the alkali-refining stage. (2) The four pesticides were removed or eliminated completely by deodorizing at temperatures usually used in commercial practice.

INTRODUCTION

Nearly 99% of the soybeans processed in Japan are imported from the United States and other countries. Postharvest application of pesticides is used to prevent losses due to pest damage during storage and transportation. The residual levels are usually much greater than those due to preharvest applications because postharvest applications are not reduced by weathering or translocation. Concern has focused on removal of the pesticides by food production processes. Removal of organochlorine pesticides from crude oil has been studied (Hashemy-Tonkabony and Soleimani-Amiri, 1976; Addison et al., 1978; Chaudry et al., 1978; Smith et al., 1968; Kanematu et al., 1974; Miyagawa, 1988). These authors concluded that the deodorizing process is important in minimizing pesticide residues in the final products. However, those studies were limited to the removal of organochlorine pesticides and contaminants (e.g., PCB). No study has dealt with pesticides, such as the organophosphorus pesticides (Figure 1) that are widely used today. Studies are needed to determine the effects of each stage of the refining process on pesticides residue levels. Residues in byproducts, which are probable sources of human foods or drugs, are also of concern. Simulated commercial processing procedures were established for study in the laboratory.

EXPERIMENTAL PROCEDURES

Apparatus. (a) A Shimadzu Model 14A gas chromatograph with an electron capture detector splitter was used. A DB-1 capillary column (30 m × 0.32 mm i.d.) with a film thickness of 0.25 μm was used with helium carrier gas at 3 mL/min and a helium septum purge of 3 mL/min. A split ratio of 20 was used. The injector and detector temperatures were 250 and 280 °C, respectively. The column oven temperature was programmed as follows: maintained at 40 °C for 1 min and then increased to 150 °C at 20 °C/min, held at 150 °C for 1 min, then increased at 4 °C/min to 210 °C, and finally maintained at 210 °C for 30 min. A 1-μL injection in the splitless mode was utilized and followed by a waiting time of 1 min. Makeup gas consisted of nitrogen at 50 mL/min.

(b) A Shimadzu Model 9A gas chromatograph with a flame photometric detector with splitter was used. A DB-5 capillary column (30 m × 0.32 mm i.d.) with a film thickness of 0.25 μm was used with helium carrier gas at 3 mL/min and a helium septum purge of 3 mL/min. A split ratio of 20 was used. The injector and detector temperatures were 250 °C. The column oven temperature was programmed as follows: maintained at 120 °C for 1 min and then increased to 150 °C at 20 °C/min, held at 150 °C for 1 min, then increased at 4 °C/min to 210 °C, and finally

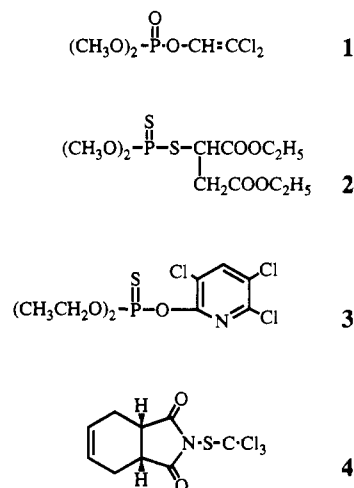


Figure 1. Structures of pesticides.

maintained at 210 °C for 10 min. A 1-μL injection in the splitless mode was utilized and followed by a waiting time of 1 min. Makeup gas consisted of nitrogen at 50 mL/min.

(c) A JEOL Model MS-DC06 gas chromatograph with mass spectrometer DX300 with splitter was used. A DB-1 capillary column (15 m × 0.53 mm i.d.) with a film thickness of 1 μm was used with helium carrier gas at 20 mL/min. A split ratio of 20 was used. The injector and separator temperatures were 230 °C. The column oven temperature was programmed as follows: maintained at 140 °C for 1 min and then increased to 230 °C at 8 °C/min and finally maintained at 210 °C for 10 min. A 5-μL injection in the splitless mode was utilized and followed by a waiting time of 2 min. EI mode was utilized at 70 eV. A JEOL GC-mass data processor was used.

(d) A Horiba B201 pH meter was used.

Reagents. (a) Pesticide standards included dichlorvos [1, *O,O*-dimethyl *O*-(2,2-dichlorovinyl) phosphate, >98%]; malathion [2, *S*-(1,2-dicarboxyethyl) *O,O*-dimethyl dithiophosphate >98%]; chlorpyrifos (3, *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, >99%); and captan [4, *N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide, >98%]. These standards were purchased from Wako Pure Chemicals Co.

(b) All organic solvents for analysis were of pesticide residue grade.

(c) Hexane for extraction was of food additive grade [mixture of *n*-hexane and pentane (1:1)].

(d) Sodium hydroxide and phosphoric acid for alkali refining were of food additive grade.

(e) Active clay for bleaching was of food additive grade.

(f) Urea, dipotassium hydrogen phosphate, and potassium dihydrogen phosphate were of analytical grade and were purchased from Wako Pure Chemicals.

(g) Hexane/acetonitrile extractions were done with mutually saturated solvents.

Samples. (a) A sample of soybeans graded U.S. No. 2 yellow soybean was obtained from the Yokohama quarantine office. Soybeans were hand-sorted to eliminate contaminants (corn, weed seeds, etc.)

(b) Flake, crude oil, alkali-refined oil, and bleached oil were donated by The Nissin Oil Mills, Ltd.

Safety Precautions. Flammable solvents, caustic reagents, and high temperature steam should be handled with care.

Preparation of Soybeans and Flakes with Pesticides. A mixture of the pesticide standards in acetone was sprayed onto the surface of the sample in a rotating flask. After 15 min of rotation, the sample was stored for subsequent processing.

Preparation of Oils with Pesticides. A mixture of pesticide standards in acetone was added to the oil in a rotating flask. The acetone content in prepared oil was less than 1%. The stirring was continued for 2 h.

Roasting. A soybean sample (150 g) at 19 °C was placed in an oven at 100 °C. The temperature of soybeans was increased to 65 °C after 45 min and was maintained automatically at 65 °C for 30 min. The soybeans were ground immediately with a Hosokawa mill. The soybeans were analyzed for moisture, water-soluble nitrogen, urease activity, and pesticide concentrations.

Extraction of Oil from Flakes. A 100-g sample of soybean flake (thickness, 0.3 mm) was added slowly over 30 s with stirring to 300 g of hexane at 55 °C. The solvent was decanted after 20 min. The process was repeated four times. Each extract was analyzed for pesticide, oil, and moisture contents.

Alkali Refining. A 1000-g sample of crude oil with an acid value of 0.69 was heated, and 25 g of water was added at 80 °C with stirring for 15 min. Then 85% phosphoric acid (0.15 g) was added to the oil, and the mixture was stirred for 1 min; twenty degree Bé (Baumé) sodium hydroxide (5.45 g) was then added (130% excess) and the stirring continued for 10 min. The mixture was centrifuged for 10 min to separate the oil from the foots. The oil was washed three times with 200 mL of water at 75 °C. The mixture was centrifuged for 5 min to separate the oil and water.

Bleaching. Alkali-refined oil (1000 g) was heated at 105 °C under reduced pressure (3–4 Torr) for 5 min. The mixture was cooled to 60 °C, and active clay (10 g) was added to the hot oil at atmospheric pressure. The temperature of the mixture was maintained at 105 °C for 15 min with stirring under the reduced pressure (3–4 Torr). The mixture was cooled to 60 °C and filtered with cotton cloth.

Deodorizing. Bleached oil (785 g) was heated at 255 °C under reduced pressure (5 Torr). Steam (total 24 g) was added to the oil for 90 min. To trap scum, the cold trap was cooled at –40 °C with an acetone–dry ice mixture.

Measurement of Moisture in Soybeans and Meal. Ground sample (10 g) was heated at 130 °C for 3 h. The weight loss was measured after cool-down in a desiccator.

Pesticide Analytical Procedure for Soybeans, Dry Soybeans, and Soy Flakes. The procedures are modifications of previously reported methods (Miyahara et al., 1992a,b). Pesticides were extracted from the ground sample (10 g) with two portions of acetonitrile (100 + 50 mL). After filtration, solvent was evaporated under reduced pressure. The residue was dissolved in 15 mL of hexane, and the pesticides were removed from the oil by extraction with three 30-mL portions of acetonitrile. The combined acetonitrile extracts were evaporated under reduced pressure, and the residue was dissolved in 5 mL of hexane. For the determination of organophosphorus pesticides, the solution was analyzed by gas chromatography without further purification. For the determination of captan, the solution was subjected to Florisil column chromatography.

Florisil PR (20 g) was packed in a chromatographic column (22 mm i.d. × 300 mm) as a slurry in hexane. The column was washed with 200 mL of hexane. The pesticides were eluted with 150 mL of ethyl acetate and hexane (1:4). The eluate was collected, and the solvent was evaporated under reduced pressure. The residue was dissolved in 5 mL of hexane.

Pesticide Analytical Procedure for Oils. Sample (2 g) was dissolved in 15 mL of hexane. The pesticides were removed from the oil by extraction with three 30-mL portions of acetonitrile. The combined acetonitrile extracts were evaporated and the

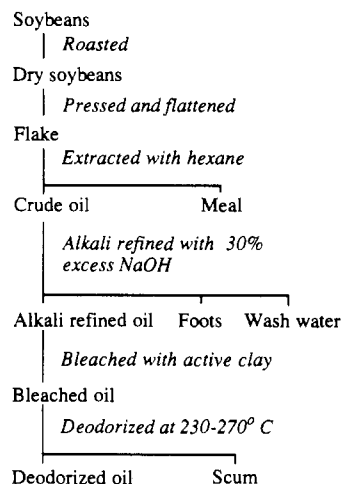


Figure 2. Laboratory oil-refining process.

Table I. Specifications for Various Stages of Soybean Oil Extraction and Refinement

process	parameter	value ^a
roasting	water content	9.5% (<10%)
	N solubility index	83 (80–85)
	urease activity	1.84 (<1.9)
extracting	oil content in extract	22.5% (18.5–20%)
	water content in extract	1.72% (<0.3%)
	oil content in meal	0.3% (2–0.6%)
	water content in meal	11.0% (10.4–13.4%)
alkali refining	acid value	0.03 (0.03–0.05)
bleaching	Lovibond index	Y20/R1.5 (Y20–35/R1.5–3.0) (5 1/4')
deodorizing	Lovibond index	Y3.0/R0.3 (Y3.0/R0.3) (5 1/4')
	acid value	ND ^b (0.03–ND)

^a The values in parentheses are specifications for commercially refined oils as reported in Miyagawa (1988). ^b Not determined.

residue was dissolved in hexane prior to Florisil chromatography as described under Pesticide Analytical Procedure for Soybeans, Dry Soybeans, and Soy Flakes.

Pesticide Analytical Procedure for Foots, Wash Water, and Scum. Each sample was extracted twice with 100 mL of ethyl acetate. The solvent was evaporated under reduced pressure. The residue was dissolved in 15 mL of hexane. This was extracted with three 30-mL portions of acetonitrile. The combined acetonitrile extracts were evaporated, and the residue was dissolved in hexane prior to Florisil chromatography as described under Pesticide Analytical Procedure for Soybeans, Dry Soybeans, and Soy Flakes.

Decomposition of Pesticides in *n*-Dodecane. Pesticides samples (200–250 mg) in a solution of xylene and *n*-dodecane (20 mL) were heated in a oil bath at 200 °C. Samples (1 mL) were taken from the solution at 0, 5, 10, 20, 40, and 80 min. These samples were diluted with hexane (200 mL) immediately after sampling, and the diluted solutions were subjected to GC–mass analysis.

RESULTS AND DISCUSSION

Simulation of Commercial Soybean Oil Refining. Various parameters were measured to confirm that the procedures in this study are comparable to the corresponding commercial refining processes (Figure 2). These parameters were measured by the standard analytical methods for oils and fats which are authorized by the Japan Oil Chemists' Society.

As shown in Table I, the products prepared by the laboratory closely approximate the specifications for commercially refined oil. The roasting process selected yielded "rare" cooked soybeans. The nitrogen solubility

Table II. Material Balance for Each Stage of Processing

material	wt, g	product	wt, g	yield, %	total yield, %
soybeans	150.0	dry soybeans	148.5	99.0	99.0
flake	100.0	crude oil	22.9	22.9	97.9
		meal	75.0	75.0	
crude oil	1000	alkali-refined oil	946.8	94.7	100.6
		wash water	633.7	4.4	
		foots	(43.7) ^a 15.1		
alkali-refined oil	1000	bleached oil	976	97.6	97.6
bleached oil	785.2	deodorized oil	777.2	99.0	99.8
		scum	6.1	0.78	

^a Weight of residue after water was removed.

Table III. Concentrations of Pesticides in Spiked Samples

sample	concentration, ppm			
	dichlorvos	malathion	chlorypyrifos	captan
soybeans	8.35	9.76	7.94	6.21
flake	10.77	6.67	6.63	5.17
crude oil	8.61	9.83	2.93	7.50
alkali-refined oil	7.94	9.00	3.04	7.03
bleached oil	8.12	8.80	3.74	5.71

index (83) and urease activity (1.84) are consistent with those of partially cooked soybeans (Miyagawa, 1988). However, this procedure is adequate to estimate the effect of roasting on pesticide residues in soybeans. The oil extraction process used in this study is more efficient than the commercial process. The oil content (22.5%) was greater than the reference values within the range 18.5–20% (Miyagawa, 1988). Moisture levels in the extracted oil and the meal were greater than the specification of <0.3%. This difference was considered unimportant because the dry commercial products will absorb moisture from the atmosphere. The deodorizing process did not effectively eliminate the coloring matter in the bleached oil. The Lovibond index matched the specified value of 3.0Y/0.3R (Miyagawa, 1988). The acid value was below the limit of detectability; therefore, the process was judged to be adequate. As discussed above, deviations from specifications were observed, but in general, each process successfully simulated the commercial refining process.

This conclusion is also supported by the material balances shown in Table II. The yields of crude, alkali-refined, bleached, and deodorized oil were 23, 95, 98, and 99%, respectively. These data are comparable with the standard results of commercial oil-refining processes (Miyagawa, 1988). Therefore, the pesticide removal efficiencies of the laboratory processes would be expected to be comparable to those of the commercial-scale processes.

Recovery of Pesticides at Various Stages in Soybean Processing. In commercial practice pesticides are applied to the raw soybeans to prevent pest damage during shipment; however, in our work we ensure that adequate residues remained after completion of the process to permit computation of the pesticide removal efficiencies. The concentrations of the pesticides are shown in Table III. To obtain homogeneous samples, special techniques were necessary. Without adequate mixing, variations in pesticides levels were excessive. As shown in Table IV, the residual pesticides in products were measured after each process. Since samples lost weight during processing, the concentrations were calculated by the bases of products weights. The recoveries are based upon the amount of added pesticides as indicated in Table III, and the results are also shown in Table IV.

Dichlorvos is easily removed by each processing step with the exception of the extraction step. Recoveries of dichlorvos in the oils were 10, 111, 1.3, 3.8, and 0% for the roasting, extraction, alkali refining, bleaching, and deodorizing, respectively. Very small amounts of the pesticide are found in the soybean byproducts (level and recovery: in meals, 0.0095 ppm, 0.03%; in foots, 1.87 ppm, 0.32%; in wash water, 0.41 ppm, 3.02%; in scum, 0.078 ppm, 0.007%). Significant pesticide reduction is achieved with every process except extraction. Since dichlorvos is a very volatile and chemically labile as well as unstable pesticide, it may be vaporized by heat and reduced pressure and/or decomposed by high temperature.

Malathion was effectively removed by the alkali-refining and deodorizing steps. Recoveries were 93.4, 86.5, 9.82, 47.0, and 0% after roasting, extraction, alkali-refining, bleaching, and deodorizing processes, respectively. Small amounts persisted in the byproducts (level and recovery: in meals, 0.015 ppm, 0.07%; in foots, 14.6 ppm, 2.25%; in wash water, 0.024 ppm, 0.15%; in scum, 0.46 ppm, 0.041%). The loss due to alkali refining may be the result of hydrolysis to give fumarate and diethylphosphorodithioic acids (Norris et al., 1954). Since a very small percentage was found in the scum, malathion may also decompose during the deodorizing process.

Chlorpyrifos was removed effectively only by the deodorizing step. Recoveries from all of the other processes ranged from 69 to 98%; therefore, low levels were found in the byproducts (meals, 0.89 ppm, 0.42%; foots, 9.14 ppm, 4.71%; wash water, 1.02 ppm, 0.43%). On the other hand, chlorpyrifos was removed efficiently from bleached oil by the deodorizing process. Deodorized oil was found to be free of the pesticide, and a small amount was trapped in scum (0.43 ppm, 0.089%).

Captan is removed effectively by roasting and bleaching with recoveries of 15 and 0%, respectively. The recoveries for the extraction, alkali refining, and bleaching varied from 82 to 92%. Small amounts of captan were found in the byproducts (meals, 0.056 ppm, 0.33%; foots, 0.089 ppm, 0.018%; wash water, 0.0023 ppm, 0.019%; scum 0.18 ppm, 0.024%). Captan may be lost during roasting due to reaction with a thiol group in the soybeans. Similar reactions have been suggested for other pesticides (Miyahara et al., 1992a,b). The alkali-refined oil retained 99% of the captan originally in the crude oil. Apparently, the hydrolysis reaction does not proceed (Wolfe et al., 1976).

Thus, the deodorizing process is effective in removing pesticide residues. This is probably due to a combination of decomposition and volatilization.

Thermal Disappearance of Pesticides (Figure 3). The deodorizing process is quite effective in reducing pesticide contamination in the oil. To understand this process, pesticides were heated at 200 °C. The concentrations of pesticides were monitored by gas chromatography with mass spectrometric detector. After 80 min, captan and dichlorvos were reduced to 5 and 15% of the initial levels, respectively. However, the chromatograms of total ion monitors revealed only a few new peaks as the intensities of the peaks due to the parent substances decreased. One degradation products of dichlorvos is dimethyl phosphate. The intensity of the dimethyl phosphate peak increased. Most of the dichlorvos decomposes into dimethyl phosphate and other products that vaporize. A small early eluting peak was observed in the gas chromatogram of the captan solution. This was identified as tetrahydrophthalimide, which is a breakdown product of captan (Wolfe et al., 1976; Cairns et al., 1983).

Table IV. Concentrations and Recoveries of Pesticides in Processed Oils

sample	dichlorvos		malathion		chlorpyrifos		captan	
	concn, ppm	recov, %	concn, ppm	recov, %	concn, ppm	recov, %	concn, ppm	recov, %
dry soybeans	0.88	10.4	9.21	93.4	7.68	98.2	0.94	15.0
crude oil ^a	56.8	110.9	27.5	86.5	26.0	82.3	20.1	82.1
alkali-refined oil	0.12	1.32	1.02	9.82	2.14	69.2	7.38	93.3
bleached oil	0.31	3.8	4.33	47.0	2.40	77.1	6.64	92.2
deodorized oil	ND ^b	0.0	ND	0.0	ND	0.0	ND	0.0

^a The concentrations were calculated by the bases of the crude oil weight. ^b Not detected.

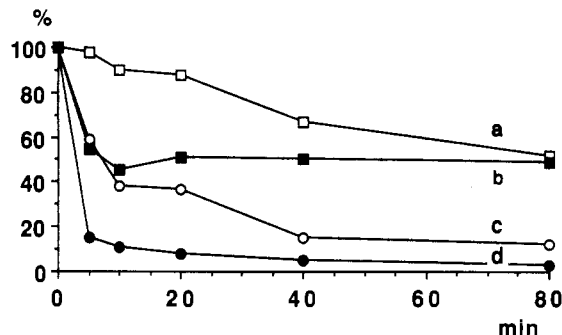


Figure 3. Thermal disappearance of pesticides: a, malathion; b, chlorpyrifos; c, dichlorvos; d, captan.

Table V. Theoretical Retention of Pesticides at Each Stage of Processing

sample	dichlorvos, %	malathion, %	chlorpyrifos, %	captan, %
soybeans	100	100	100	100
dry soybeans	10.4	93.4	98.2	15.0
crude oil	11.6	80.8	80.8	12.3
meals	0.0029	0.065	0.41	0.051
alkali-refined oil	0.153	7.34	55.9	11.46
foots	0.038	1.82	3.81	0.0022
wash water	0.035	0.125	0.822	0.0023
bleached oil	0.0058	3.73	43.1	10.6
deodorized oil	0	0	0	0
scum	0.00000043	0.0015	0.0384	0.0025

Some of the captan may decompose to volatile products that disappear from the solution.

Malathion and chlorpyrifos are more stable than dichlorvos and captan. After 80 min, 50% of the malathion and chlorpyrifos still remained. Those compounds have been reported to be unstable and subject to thermal decomposition (Saito et al., 1981), but as described above the pesticides are not retained in the deodorized oil and scum. These results show that it is difficult to remove these pesticides thermally without steam and vacuum. Therefore, the steam in combination with heat and vacuum effectively removes pesticides from the bleached oil.

Retention of Pesticides. Using the results in Tables II and IV, the residues of pesticides in each step of the oil-refining processes are calculated; the results are shown in Table V. Overall recoveries of the pesticides are quite low. The final oil product is essentially free of pesticides; however, the byproducts, such as meals, foots, and scum, do retain small amounts of pesticides. Byproducts are used as sources of human foods and drugs. For this reason, it is necessary to continue to monitor pesticides in raw soybeans.

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