Some Radiochemical Experiments on Minor Constituents in Soybean Oil[1]

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Abstract

A ^{14}C-labeled high molecular weight hydrocarbon and an insecticidal compound were added as minor constituents in soybean oil samples. Liquid scintillation counting was used to assay the radioactivity of the oil preceding and after laboratory simulations of commercial processing procedures (bleaching and deodorization). Radiochemical techniques were found to be highly sensitive and quantitative and detection was unaffected by chemical modification or decomposition of the parent compound. Labeled (^{14}C) benzo(*a*)pyrene was retained primarily by the oil during extraction, filtering, solvent stripping, deodorization and treatment with AOCS bleaching earths. Treatment of the oil with activated charcoal effected removal of this hydrocarbon. Bleaching was ineffective in removing added ^{14}C-endrin from the oil but a deodorization using specific conditions of temperature (250 C), time (2 hr), and pressure (4.5 mm) removed this constituent.

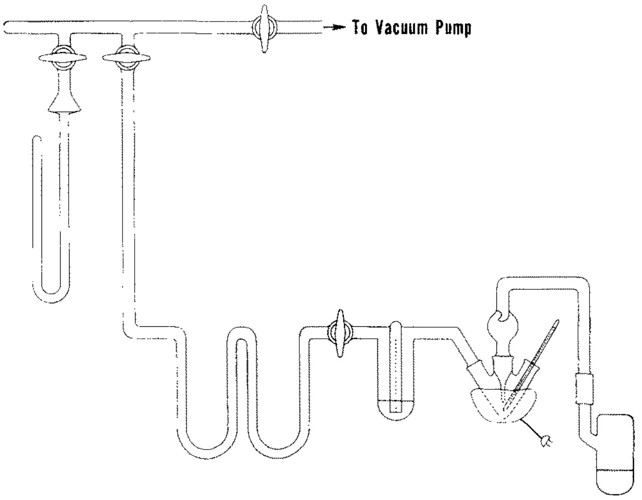

FIG. 1. Vacuum manifold modified to simulate commercial deodorization procedures: (A) Source of steam, (B) 300 C thermometer, (C) 50 ml pear-shaped flask, (D) Glas-Col mantle (E) sealed end monometer, and (F) traps.

Introduction

Radiochemical techniques offer some unique advantages for experimentally evaluating the efficiency of oil processing procedures for removal of minor constituents. By using materials with high specific activity, detection by scintillation methods of the radioactive emissions of exceedingly small amounts added to an oil often surpasses the sensitivity achieved by other means. Also, chemical modification or decomposition of the constituent which can confuse other detection methods, in general, will not effect detection based on the radioactivity of the constituent.

The presence of polycylic aromatic hydrocarbons as minor constituents in a variety of vegetable oils has been reported by several investigators (2,6–8). Bruce et al. (3) showed that insecticidal residues accumulated in soils were translocated to seeds of high oil-bearing crops, specifically soybean and peanut. Little has been reported concerning the efficiency of commercial processing procedures in removing these constituents. Gooding (4) reported on the effective removal of several chlorinated organic pesticides from cottonseed oil; however, he included little information concerning conditions of deodorization. The present paper reports results of laboratory simulations approximating commercial practice, indicating ineffective procedures and those techniques which are effective in removal of minor constituents and the specific conditions required.

Experimental Procedures

Materials and Equipment

^{14}C-Endrin. ^{14}C-Endrin was obtained from Nuclear Research Chemicals, Inc. It was purified by thin layer chromatography (TLC) on silica gel with cyclohexane as the mobile solvent. The purified ^{14}C-endrin had a specific activity of 0.84 mc/mM.

Benzo(a)pyrene. 8,9-^{14}C-Benzo(*a*)pyrene was supplied by Baird Atomic, Inc. TLC indicated that the sample was chemically and radiochemically pure; its specific activity was 3.23 mc/mM.

Soybean Oil and Flakes. Soybean oil (IV 134.6, 7.6% linolenic acid), alkali refined and bleached, was purchased from A. E. Staley Co. It was deodorized before use. Soybean flakes were prepared from hulled beans in a flaking mill at this Laboratory.

Radiochromatogram Scanner. A Packard Model 7200 radiochromatogram scanner provided a radiochemical assay of thin layer plates.

Scintillation Counter. Aliquots of oil samples (50–150 mg) labeled with radioactive constituents were dissolved in 15 ml of scintillation solvent [4.0 g 2,5-

TABLE I

Soybean Oil-Benzo(*a*)pyrene
Radioactive Analysis of Products From Soybean Flakes

Sample	Vol., ml	Total activity, μc	Retained, %	Specific activity, μc/ml	Concentration of benzo(*a*)pyrene in oil, ppm
Hexane	3000	12.3	......	4.1×10^{-3}	0.0
Miscella solution	1970	10.8	88.0	5.5×10^{-3}	.....
Stripped oil	80.7	8.5	69.5	105.3×10^{-3}	10.3
Bleached oil	51.5	5.3	43.0	102.7×10^{-3}	10.0

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TABLE II

Soybean Oil-Benzo(α)pyrene Deodorization: Radioactive Analysis

Deodorization conditions			Specific activity, μc/mg	Concentration of benzo-(α)pyrene, ppm
Temp., °C	Time, hr	Press, mm (Hg)		
200	0	2	4.13×10^{-4}	50.5
	1		3.18×10^{-4}	38.1
	2		3.23×10^{-4}	39.5
	3		3.27×10^{-4}	40.0
	4		3.17×10^{-4}	38.0
250	0	5	7.93×10^{-4}	97.0
	1.5		5.72×10^{-4}	69.2

TABLE III

Soybean Oil-Benzo(α)pyrene Bleaching With Activated Charcoal: Radioactive Analysis

Sample wt., g	Total activity, μc	Before bleaching		After bleaching	
		Specific activity, μc/mg	Conc., ppm	Specific activity, μc/mg	Conc., ppm
9.38920	5.42	5.77×10^{-4}	43.1	1.79×10^{-6}	0.14

diphenyloxazole (PPO) per liter of toluene] and assayed in a Packard Model 314-X Tri-Carb scintillation counter.

Deodorization. High-vacuum steam deodorization was simulated in the all glass equipment diagrammed in Figure 1. The flask is heated by a Glas-Col mantle and is equipped with a thermometer and a sealed end manometer.

Procedure and Data

Benzo(α)pyrene. A solution of ^{14}C-benzo(α)pyrene (12.3 μc) in 3 liters of hexane was prepared. Soybean flakes (400 g) were extracted four times by stirring them with 600 ml of this solution at 30 C for 10 min. Each miscella was drawn off the flakes through a Buchner funnel and the miscella solutions were combined. The solvent was removed on a rotary evaporator. The oil was stirred for 10 min with 6.0% AOCS bleaching earth at 120 C. Assay for radioactivity was performed after each processing step by liquid scintillation counting. The results of these assays are presented in Table I.

Refined soybean oil (10 g) was placed in the laboratory deodorizer. ^{14}C-Benzo(α)pyrene (5.0 μc) was added as a contaminant to the oil. The oil was heated to 200 C and steam was introduced at a pressure of 1–3 mm (Hg). Distillate was collected in a liquid nitrogen trap for a 1 hr period. At the end of each hour nitrogen was bled into the system, and duplicate samples were taken from the oil for assay. This procedure was repeated at hourly intervals for a total deodorization time of 4 hr. A second sample was deodorized at 250 C, 5 mm (Hg) for 1.5 hr. Steam flow rate for these experiments was about 27% per hour by weight of oil. Radiochemical assay results are shown in Table II.

Soybean oil (10 g) was placed in a pear-shaped flask with benzo(α)pyrene (5.42 μc) and Darco-activated charcoal (0.4773 g). After the flask was fitted with a stirrer and nitrogen inlet, the equipment was immersed in an oil bath at 90–100 C. Stirring at this temperature was continued for 30 min, followed by filtration on a coarse fritted disk. Radioassay was performed before and after treatment. Results are shown in Table III.

^{14}C-Endrin. Commercial bleaching procedures were evaluated for removal of ^{14}C-endrin from soybean oil. Samples (1–2.5 g) of soybean oil with 0.5–1.0 μc of added ^{14}C-endrin were treated with two different bleaching agents by stirring at 120 C for 5 min; these agents were 6.0% AOCS bleaching earth and 6.0% AOCS bleaching earth plus 0.5% activated carbon. Oil was assayed by liquid scintillation counting before and after bleaching (Table IV).

Five samples of soybean oil (10 g) with various concentrations of ^{14}C-endrin added were deodorized in the laboratory system. Temperature of deodorization was 200–250 C, pressure 1–4.5 mm (Hg), and total time of deodorization 2–5 hr. Steam flow rate was about 30% per hour by weight of oil. The oil was assayed before and after deodorization as described previously (Table V).

Discussion and Results

The use of radioactively labeled materials to simulate constituents in oils provides an unusual and independent approach to the evaluation of commercial processing procedures. Involved techniques of isolation and analysis are not required to determine residual material in the oil. Direct liquid scintillation assay of oil samples provides a sensitive quantitation of the amount of constituent remaining. Available high specific activity materials offer a limit of detection for trace constituents which exceeds that reported by Biernoth and Rost (2) (0.5 μg kg^{-1} of oil).

During the extraction of soybean flakes, benzo(α)-pyrene, which Gräf (5) reports is synthesized by plants, is retained by the soybean oil. The results of the assays as shown in Table I indicate that this constituent is not removed with the solvent nor absorbed on AOCS bleaching earth. Deodorization employing even the most extreme conditions of temperature, vacuum and time is also ineffective as a technique for removal of the high molecular weight hydrocarbon. Treatment with activated charcoal, as reported by Biernoth and Rost (2) working with coconut oil, is confirmed as effective for removal. This level of carbon is somewhat above that used commercially, as noted in "Bailey's Industrial Oil and Fat Products" (1). Two tenths per cent of carbon is about the upper limit. These experiments indicate that to ensure the removal of benzo(α)pyrene commercial processing of soybean oil must include treatment with activated carbon.

The insecticidal component is not affected by

TABLE IV

Soybean Oil-Endrin Bleaching: Radioactivity Analysis

Sample[a]	Wt., g	Total activity, μc	Before bleaching		After bleaching	
			Specific activity, μc/mg	Conc. endrin, ppm	Specific activity, μc/mg	Conc. endrin, ppm
A	2.68	1.20	4.48×10^{-4}	204	4.30×10^{-4}	196
B	2.31	1.04	4.50×10^{-4}	206	4.34×10^{-4}	198
A	0.98	0.53	5.41×10^{-4}	244	5.22×10^{-4}	238
B	0.93	0.50	5.32×10^{-4}	243	5.24×10^{-4}	239

[a] Bleaching conditions: 120 C, 5 min and 6% AOCS Natural bleaching earth (B had an additional 0.5% activated carbon).

TABLE V

Soybean Oil-Endrin Deodorizations: Radioactive Analysis

Experiment	Deodorization conditions			Soybean oil wt., g	Total activity, μc	Specific activity, μc/mg	Conc. endrin, ppm	Retained, %
	Temp., °C	Time, hr	Hg, mm					
1	210	0	1	9.38	5.250	5.60×10^{-4}	256.0	
		3			4.440	4.70×10^{-4}	203.0	79.3
2	200	0	3	9.14	0.073	0.76×10^{-5}	3.64	
		2.5			0.051	0.47×10^{-5}	2.12	58.3
3	210	0	1	9.90	0.110	1.10×10^{-5}	5.11	
		4			0.030	0.30×10^{-5}	1.42	27.8
4	210	0	1	10.53	0.082	0.78×10^{-5}	3.52	
		5			0.019	0.18×10^{-5}	0.82	23.6
5	250	0	4.5	10.22	0.080	0.79×10^{-5}	3.58	
		2			0.003	0.032×10^{-5}	0.15	4.2

bleaching either with AOCS bleaching earths or activated charcoal added to the bleaching earth. Moderate temperature deodorization conditions, (Expt. 1 through 4) which are used by industry, are only partially effective in removing the ^{14}C-endrin. High temperature conditions of deodorization (Expt. 5) reduce the level of insecticide constituent to less than 0.15 ppm about 96% effective removal. Deodorization of soybean oil at 250 C, 4–6 mm (Hg) for 1.5 to 2 hr is recommended to ensure removal of this constituent.

The results reported in this paper complement and broaden those reported by Smith et al. (9) who used conventional techniques of insecticide detection and a limited range of deodorization conditions.

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