Trace Elements in Edible Fats. VIII. Soybean Oil "Demetalization" with Cation Exchange Resins[1]

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Abstract

The effects of different solvents, degree of dilution, type of resin, and temperature were studied to determine the optimum conditions for "demetalization" of soybean oils with cation exchange resins. Prooxidant metals in crude soybean oils in acetone or *n*-hexane solutions can be effectively removed when passed through columns of a strong cation exchange resin.

Introduction

PREVIOUSLY (1,2,3) it has been shown that with the use of cation exchange resins, traces of prooxidant metal can be removed from olive oil. The iron retention by the resin was so complete that we were able to develop a method for the determination of iron in the oil without the conventional ashing technique. The demetalization process gives the olive oil a highly superior stability. This is to be expected because of the role played by the metals in autoxidation of the unsaturated fatty acids.

This paper describes the application of the demetalization technique to soybean oils. A following paper (4) will describe the observed effects on the oxidation and flavor stabilities as a consequence of partial demetalization of those oils. The technique is to pass an acetone solution of the oil through a column of a strongly acidic resin in H^+ form, with recovery of the oil by evaporating the solvent. Optimal conditions for the demetalization of soybean oil have been determined. The various conditions studied were the effects of different solvents, type and volume of resin, degree of dilution, and temperature.

Materials and Methods

All studies were made on commercial samples of crude degummed oils because of the higher metal content of these oils as compared to refined oils. Three samples of oils described as B-1D, B_2 and No. 837 were used. See Table I.

Columns. Four types of conventional glass columns, with stoppers at the lower end, were used. Column A: 15 × 300 mm; B: 15 × 560 mm; C was the same size as B, but had a water jacket to provide different temperatures. Column D: 43 × 560 mm.

Resins. Amberlite CG-120 type I and Dowex 50 W × 8, 100–200 mesh was used.

Solvents. Acetone for chromatography (Merck) and isopropyl alcohol were used without further purification. *n*-Hexane was redistilled.

Acid and peroxide values were determined by AOCS Official Methods (Ca 5a-40 and Cd 8-53). Trace metals were determined by the method of Vioque and Villagrán (5). This is based on the extractive enrichment with organic reagents and chloroform, followed by spectral analysis using beryllium as internal standard. Spectrographic analysis was made on a Hilger E 498.

Procedures

General demetalization technique. The column (A)

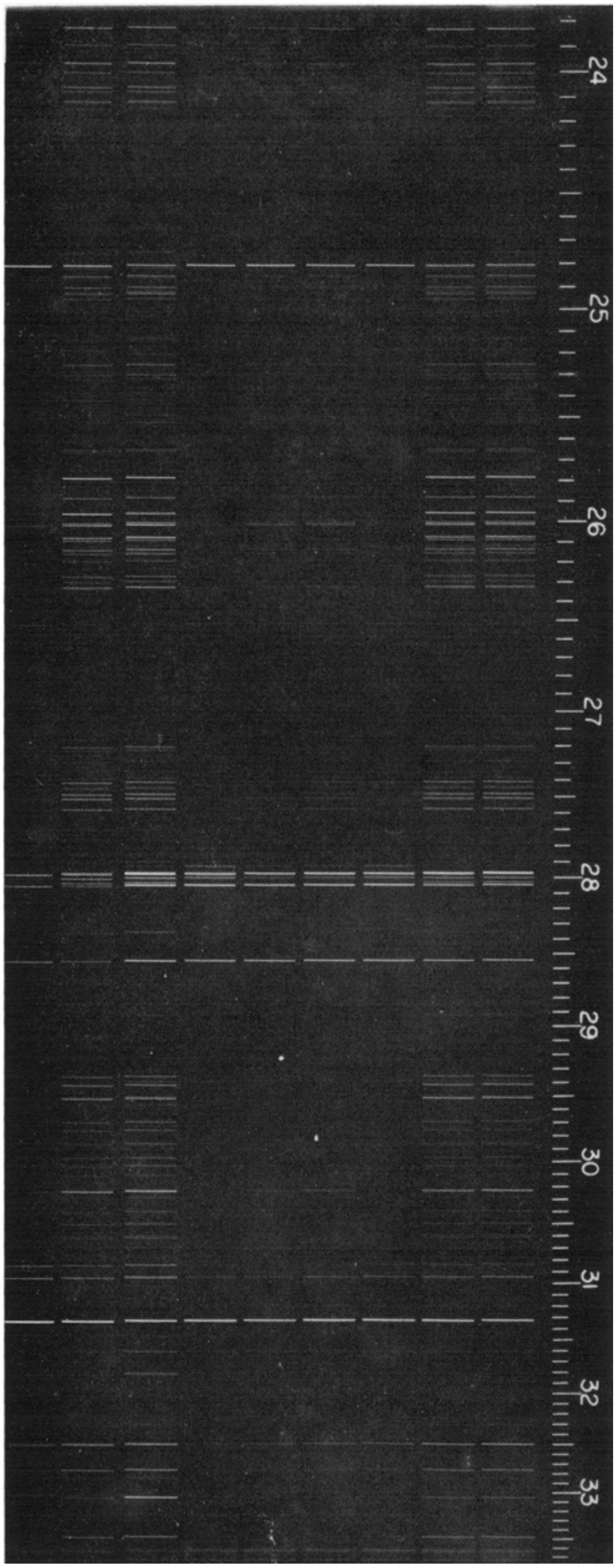

FIG. 1. Example of crude soybean oil demetalization. Order of spectra: Wavelength scale; metal content of original oil (spectra 1,2, and 3 from duplicate samples and blank); metal content of demetalized oil (spectra 4,5, and 6); retained metals by the exchange resin (spectra 7,8, and 9).

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TABLE I

Characteristics of the Soybean Oils

Oil	A.V. mg KOH/g	P.V. meq/kg	Metal content ppm			
			Fe	Mn	Cu	Zn
B-1-D	0.6	1.4	1.19 ± 0.18	0.255 ± 0.028	0.085 ± 0.012	1.23 ± 0.38
B-2	1.5	20.0	2.82 ± 0.42	0.225 ± 0.025	0.028 ± 0.004	1.72 ± 0.53
837	0.8	14.7	0.88 ± 0.13	0.281 ± 0.031	0.019 ± 0.003	1.16 ± 0.36

was filled with a slurry of Amberlite CG-120, type I, resin in deionized water to give a 15 cm packing. The column was washed upward with deionized water to eliminate the finest particles of resin and occluded air. This done, the water filling the column was allowed to filter out the column through the bottom stopcock until the resin settled down evenly. When the water level was 1 cm at the top of the column, 50 ml 3*N* HCl were added to convert the resin to the hydrogen form, the water flow was adjusted to 0.5 ml/min. The column was then washed with ca. 100 ml of deionized water, until the eluate was neutral and negative to Cl^-. It was then washed with acetone (150 ml) until the eluated acetone was miscible with oil.

Fifty g of oil were weighed in a 250 ml conical flask and dissolved in 50 ml acetone. This was poured into the column and the flow adjusted to 1 ml/min. The column was then washed with ca. 40 ml acetone to extract all the oil. Spot checks on the eluate were made by placing a drop on cigarette paper. All oil was assumed to be extracted when the acetone did not give an oil spot on the paper.

Recovery of the metals. Acetone was removed from the column by washing with 120 ml deionized water until the eluate gave negative reaction to acetone (6). The metals were extracted with 50 ml 3*N* HCl (flow of 0.5 ml/min). The column was washed with 10 ml water, and the washings were added to the 50 ml extract. The metals were determined in the total extract. The oil was recovered from the acetone solution in a rotatory evaporator (temp below 40C).

TABLE II

Reproducibility of Demetalization Technique. Oil B-1-D. Amberlite CG-120-I (H^+). Solvent: Acetone. 1:1. Temp 20C

	Retained metals ppm			
Column	Fe	Mn	Cu	Zn
1st	1.06	0.212	0.072	1.16
2nd	0.87	0.210	0.067	1.11
3rd	0.88	0.248	0.065	1.05
4th	0.85	0.248	0.081	1.39
5th	0.78	0.252	0.082	1.40
6th	0.97	0.250	0.088	1.30
Avg	0.90	0.237	0.076	1.24
Typical deviation	0.10	0.020	0.009	0.15
Variation coefficient	10.9	8.4	12.1	12.1

TABLE III

Influence of Solvent, Dilution, and Resin Volume. Oil B-1-D. Amberlite CG-120-I (H^+). Temp 20C

Column	Solvent	Dilution	Resin Volume	Retained metals (ppm)			
				Fe	Mn	Cu	Zn
1st	Acetone	1:1	30 ml	0.56	0.240	0.079	1.15
2nd	Isopropyl Alcohol [a]	1:3	30 ml	0.07	0.024	0.014	0.22
3rd	Acetone	1:3	45 ml	0.20	0.091	0.031	0.58
4th	Isopropyl Alcohol [a]	1:1	45 ml	0.11	0.030	0.028	0.38

[a] With 2% chloroform to solubilize the oil.

TABLE IV

Influence of Type of Resin, Solvent and Temp (Factorial Design). Oil B-2, Resin Volume, 30 ml. Dilution 1:1

Solvent	Retained Metals ppm	Resin			
		Amberlite CG-120-I, H^+		Dowex 50W × 8, H^+	
		10C	30C	10C	30C
Acetone	Fe	1.43	2.08	1.48	1.36
	Mn	0.201	0.220	0.194	0.156
	Cu	0.020	0.023	0.031	0.016
	Zn	1.15	2.12	1.63	1.42
Hexane	Fe	0.82	1.22	0.57	0.93
	Mn	0.155	0.220	0.184	0.208
	Cu	0.019	0.022	0.017	0.020
	Zn	1.48	1.67	0.66	1.36

With isopropyl alcohol as solvent, the volumes handled in every step were the same as those used with acetone. *n*-Hexane required a drying step following water washing, because of its immiscibility with water. This was done by passing ca. 75 ml ethyl alcohol until eluates were miscible with hexane. *n*-Hexane was passed until miscibility with oil was observed. Where the oil sample in hexane was passed through the column, 50 ml fresh hexane was added to extract the residues of oil, followed with ca. 50 ml ethyl alcohol until the eluate was miscible with water.

Reproducibility was studied with 9 columns of type A. Fifty g each of the oil B-1-D was analyzed on 6 columns. The other 3 were used as control blanks. Acetone was used as the solvent in a dilution 1:1 on 30 ml resin Amberlite CG-120-1-(H). (Table II).

Influence of type of resin, solvent, and temperature. A latin square (7) was statistically planned to study the effects of solvents, dilution (1.1; 1:3) and volume of resin (30 ml; 45 ml). The resin was Amberlite CG-120-I (H+), which was efficacious in previous work with olive oils. (Table III).

In another experiment planned in a factorial design, the effect of solvent (acetone, hexane), type of resin (Amberlite CG-120-I; Dowex 50 W × 8) and temp (10C, 30C), was studied. (Table IV).

Results and Discussion

In previous studies (8) we observed that both the resins (H^+ form) used in this study darkened considerably when in contact with acetone above 15C over extended periods. Eluated acetone was also colored but the metal retention capacity was not affected. In the present studies, precautions were taken not to leave the resin in contact with acetone above 15C for a long time.

The interpretation of the results on Table III, by analysis of the variance, is done as follows. The error

TABLE V

Confirmation of Demetalization Obtained with Acetone-Amberlite Treatment. Oil B-1-D

	Metal content ppm)			
	Fe	Mn	Cu	Zn
Original Oil	1.19	0.255	0.085	1.23
Demetalized Oil	0.23	0.000	0.017	0.00
Eluates from the resin	1.01	0.228	0.067	1.53

TABLE VI

Influence of Resin Type and Solvent. Confirmation with Duplicate Treatments. Oil B-2. Volume of Resin, 30 ml. Dilution, 1:1. Temp 20C

Solvent	Retained metals ppm	Resin	
		Amberlite CG-120-I, H^+	Dowex 50W × 8, H^+
Acetone	Fe	1.93 –1.51	1.17 –1.53
	Mn	0.255–0.216	0.168–0.196
	Cu	0.033–0.024	0.009–0.015
	Zn	2.30 –1.55	1.28 –1.27
Hexane	Fe	1.38 –1.31	0.31 –0.42
	Mn	0.204–0.224	0.178–0.194
	Cu	0.009–0.018	0.012–0.020
	Zn	1.23 –1.37	1.50 –1.36

TABLE VII

Effects of Successive Treatments Hexane-Amberlite. Oil B-2. Dilution 1:1. Temp 20C

	Retained metals (ppm)							
	Fe		Mn		Cu		Zn	
	1	2	1	2	1	2	1	2
Column A (15 cm)								
First Treatment	1.38	1.31	0.204	0.224	0.009	0.018	1.23	1.37
Second treatment	0.67	0.38	0.047	0.026	0.012	0.008	0.21	0.04
Total retention	2.05	1.69	0.251	0.250	0.021	0.026	1.44	1.41
Column B (30 cm)								
First Treatment	1.90	1.14	0.300	0.310	0.019	0.011	1.88	1.66
Second treatment	1.02	0.99	0.002	0.002	0.003	0.000	0.00	0.00
Total retention	2.92	2.13	0.302	0.312	0.022	0.011	1.88	1.66
Incinerated demetalized oil	0.21	0.08	0.000	0.000	0.012	0.010	0.09	0.00

taken is the one calculated as described above and from the variation coefficient the typical deviation which corresponds to the average value of the four values of the latin square for the metal studied is calculated, which presupposes that the coefficient variation does not change within this concentration range. It was concluded that the influence of solvent and dilution is significant in favor of acetone (1:1), and the influence of resin volume for iron and manganese, but not for copper and zinc, is significant in favor of the small volume. The conclusion that a larger column achieved a lower retention of iron and manganese is difficult to explain. The passing of additional 50 ml 3*N* HCl through the column showed that the metal elution was complete. The only possible explanation could be the presence of a real interaction between solvent and dilution which, in the planned latin square, would be confounded with the influence of resin volume.

Conditions belonging to the first column are therefore considered favorable. Under these conditions, an assay of demetalization was carried out with duplicate samples of soybean oil B-1-D. Spectra obtained with the original oil; the demetalized oil; and the eluates obtained with HCl from the columns, are shown in Figure 1. (Table V).

Statistical interpretation of the results on Table IV shows significant influence of the solvent, in favor of acetone in the case of iron. To confirm the results, a new factorial experiment was planned, with duplicate treatment at room temp, as operating temp did not appear to be critical. Results (Table VI) confirm that the combination, acetone-Amberlite, is the most efficacious. In the statistical analysis of the data of this factorial design (Table VI) the mean square corresponding to the duplicate was used as error.

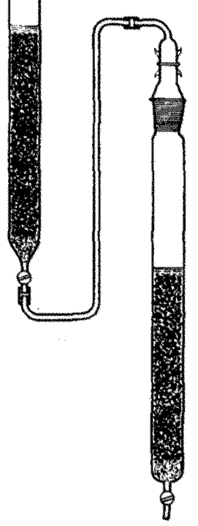

FIG. 2. Exchange columns mounted in series for demetalization of 400 g crude soybean oil.

TABLE VIII

Incomplete Retention of Iron From Oil No. 837.
Treatment: Hexane-Amberlite

	Metal content (ppm)			
	Fe	Mn	Cu	Zn
Original Oil No. 837	0.88	0.281	0.019	1.16
No. 837 passed once	0.78	0.008	0.005	0.00
No. 837 passed twice	0.40	0.000	0.002	0.08
Total demetalization %	55	100	90	93

Although the results obtained show the treatment acetone-Amberlite as favorable, authors realized that *n*-hexane offers considerable advantages. Commercially the oil is extracted with *n*-hexane and the resin may stay in contact with the solvent indefinitely. Therefore, the hexane-Amberlite treatment was studied in detail. The effects of eluting *n*-hexane solutions twice through column A (15 cm resin), once through column B (30 cm resin), and twice through column B were studied. The oil used was B-2. See Table VII.

Using a second *n*-hexane-Amberlite treatment in column A, demetalization is obtained similar to that with a single acetone-Amberlite treatment. Effective demetalization is also obtained with a single treatment in column B. Hexane-Amberlite was therefore adopted for further studies. Two columns of type D were mounted in series (Fig. 2) to demetalize 400 g oil. When this was used with oil No. 837, satisfactory demetalization resulted with respect to manganese, copper, and zinc; but iron was not effectively retained. (Table VIII). These tests were repeated with similar results, which indicates a different behaviour of this oil as compared to soybean oil B-2, as far as the capacity of interchange of iron is concerned. Possibly the iron in the oil was in a form not retainable by the resin. We have insufficient data to offer a satisfactory explanation.

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