with log concn over a wide anionic concn range.

Nonionic surfactants lower mobility of both triolein and glass, thus have slight cationic character. Significant inflection points and minima at cmc were found in mobility-concn curves.

The cationic agent CTAB produced high positive charge and zeta potentials on triolein and glass. Inflection points were noted at cmc and, as with nonionics, mobility is relatively constant above cmc.

Surfactant-builder combinations yielded mobilities and zeta potentials essentially equal to those for builder alone; no synergism was evident. Oil droplets sorb surfactants through their hydrocarbon chains, and polar groups extending into the aqueous phase determine the sign and degree of charge. Glass has little tendency to adsorb negative ions, even those which are surface active. Surfactant cations are strongly adsorbed by glass and the adsorption apparently occurs with the positively charged polar group attaching to the glass. Nonionics behave like weak cationic agents, apparently attaching to glass through protonated ether oxygens which have a slight positive charge. Electrokinetic effects are not necessary and sufficient causes for fatty soil removal though they may be contributing factors, especially for ionic agents. The electrical properties are probably more important in suspending action and in preventing soil from redepositing on a surface once removed.

REFERENCES

1. Harris, J. C., Textile Res. J., *28*, 912 (1958).
2. Kruyt, H. R., "Colloid Science," Elsevier Publishing Co., 1952.
3. Adamson, W. A., "Physical Chemistry of Surfaces," Interscience Publishers, Inc., New York, 1960, Ch. IV.
4. Powell, B. D., and A. E. Alexander, Can. J. Chem. *30*, 1044 (1952).
5. Karpova, I. F., Uchenye Zapiski Leningrad. Gosudarst. Univ. im. A. A. Zhdanova No. 150, Ser. Khim. Nauk No. 10, 87–98 (1951).
6. Bikerman, J. J., "Surface Chemistry," Academic Press, Inc., New York, 1958, p. 319.
7. Powney, J., and L. J. Wood, Trans. Faraday Soc. *36*, 57 (1940); *Ibid.*, 420 (1940).
8. Dean, R. B., and W. J. Dixon, Anal. Chem. *23*, 636 (1951).
9. ASTM Manual on Quality Control of Materials, Special Technical Publication 15-C (1951).

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Soybean Unsaponifiables: Chromatographic Investigation of Shell Drain Condensate from a Commercial Deodorizer

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Abstract

The shell drain condensate from a commercial soybean oil deodorizer was analyzed for nonpolar unsaponifiable constituents by liquid-liquid partition chromatography. A procedure for obtaining large quantities of these nonpolar constituents for flavor studies is described, and data on extraction techniques, purification, and analyses are presented. High-purity soy sterols can be obtained as a by-product.

Introduction

PREVIOUS work (4) indicated that the nonpolar constituents of soybean unsaponifiables, when added to freshly deodorized oils, were responsible for some of the oxidative flavor deterioration. Since unsaponifiables represent only about 1% (4,8) of soybean oils, further investigation of nonpolar consituents was hampered by the low concentration, usually less than 15%, in the total unsaponifiables. Adequate characterization and organoleptic study of nonpolar constituents require more sample than is ordinarily available from the usual quantitative procedure for the determination of unsaponifiables. This difficulty prompted an investigation of other starting materials for laboratory work.

Procedures

A commercial soybean oil shell drain condensate (SDC) was analyzed as a more concentrated source of unsaponifiables. SDC represents 7–10% of the deodorizer loss and contains constituents of low volatility that collect on the outer shell of a tray-type deodorizer (2). Such a deodorizer collects distillates of low volatility by allowing them to drain to the bottom where they are removed from the system as a green or brown oil.

Volatile and nonvolatile fractions were obtained upon distillation of the SDC in an Asco 50 Rota-Film molecular still at 200C and 4 μ. SDC, its fractions, and the unsaponifiables obtained from these fractions were analyzed by liquid-liquid chromatography, on 50-g silicic acid columns with 16% methanol (by wt of SiO_2) as the mobile phase and 2% methanol (by

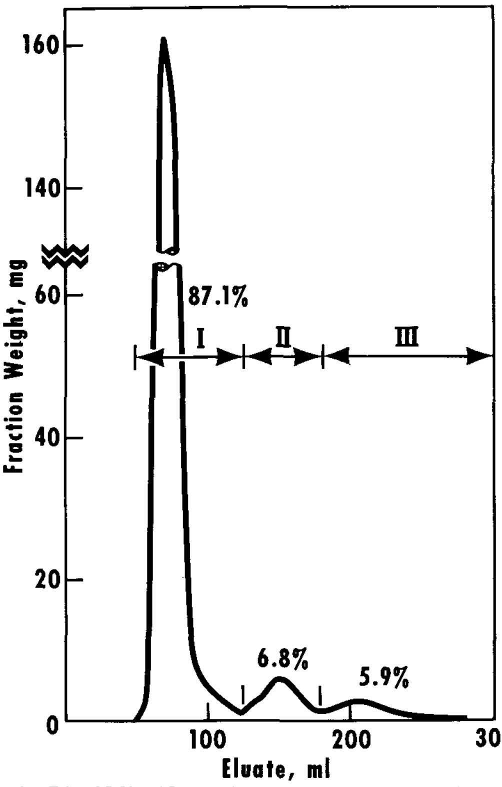

FIG. 1. Liquid-liquid partition chromatogram of shell drain condensate (SDC).

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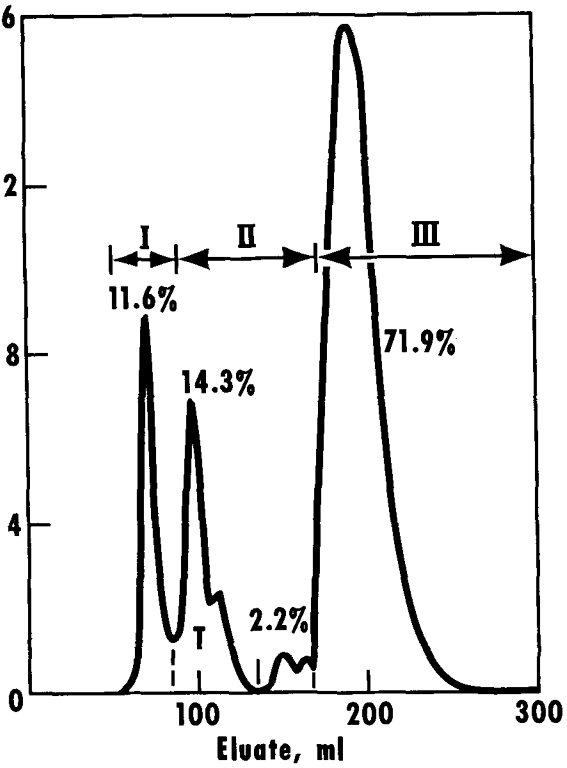

Fig. 2. Liquid-liquid partition chromatogram of SDC unsaponifiables.

vol) in benzene as the immobile phase. Eluted fractions were freed of solvent and weighed. Weights were plotted versus elution volume to obtain the chromatograms. Liquid-partition chromatography (LPC) and thin-layer chromatography (TLC) were conducted as described previously (3,4). Gas-liquid chromatographic analysis of the various fractions was obtained with a 6-ft silicone rubber column and allowed preliminary comparisons of SDC unsaponifiables with those obtained from soybean oil in the conventional manner.

Experimental

Whole nonsaponified SDC contained 87.1% nonpolar components (Fraction I in Fig. 1). Further analysis showed Fraction I to be composed primarily of saponifiable materials because esters eluted in the first fraction during chromatographic separation. Free sterols (Fraction III), whose elution position was determined by the Liebermann-Burchard test, comprised 5.9% of the total SDC.

The SDC contained 23.0% unsaponifiables, obtained as a light-brown dry solid after saponification with 50% alcholic alkali (4). Usually soybean unsaponifiables are brown, waxy, semisolids that differ physically from those in SDC. Apparently the SDC unsaponifiables contain considerably more than the normal amount of sterols, which lighten the color and increase crystallinity. Purified soy sterols are dry white solids.

Chromatography of the SDC unsaponifiables, Figure 2, indicated 11.6% Fraction I (nonpolar unsaponifiable components) and 71.9% Fraction III (polar components primarily sterols). Part T of Fraction II constituted 14.3% of the unsaponifiables and was identified by LL, TL, and GL chromatography as tocopherols. SDC seems to be a rich source of these natural soybean oil antioxidants since it contains over 3,000 mg tocopherol per 100 g. The usual concentration in soybean oil ranges from 74–280 mg per 100 g (6).

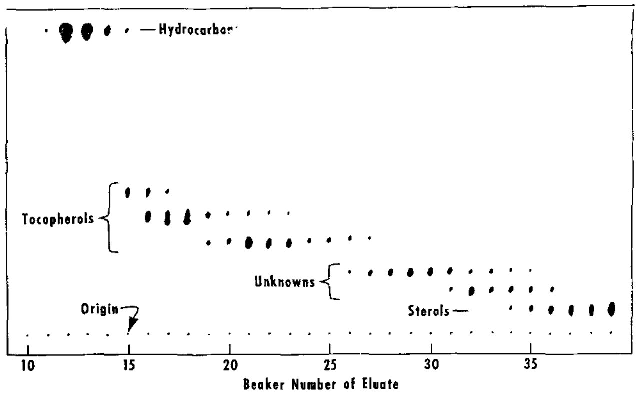

Fig. 3. Thin-layer chromatogram of fractions obtained from liquid chromatographic separation of SDC unsaponifiables. Beaker numbers correspond to the elution volume shown in Figure 2; each beaker contains 5 ml.

Figure 3 shows results obtained by TLC on fractions from the liquid column, Figure 2. Each beaker number corresponds to 5 ml of eluate from the silicic acid column; a comparison of the two figures shows the fractionation obtained. Mallinkrodt silicic acid was also used for TLC, which were developed with 10% diethyl ether solution in pentane-hexane. After drying, the plates were sprayed with 50% sulfuric acid and heated until charring developed the spots. The high resolution indicates that hydrocarbons, tocopherol isomers, and other materials in the unsaponifiables separate well on the silicic-acid liquid column.

The light color and dry appearance of the SDC unsaponifiables indicate that a large proportion of sterols could be expected. Saponification and acidification of SDC (no ether extraction of soaps) allow conversion of all glyceride and ester materials to free fatty acids. A liquid chromatogram of this acidified material (Fig. 4) permits estimating nonpolar constituents, free sterols, and sterol esters in the unsaponifiables on the basis of whole SDC. A simple calculation

15.3%	total sterols (Fraction III, Fig. 4)
– 5.9%	free sterols (Fraction III, Fig. 1)
9.4%	sterols as esters liberated by saponification

shows that 61.4% of the sterols exist as esters. Fraction III, from typical unsaponifiables extracted from soybean oil, usually is two or three times more than Fraction I. With SDC unsaponifiables, Fraction III outweighs Fraction I by about 6.5–1 (Fig. 2). This increase indicates a possible loss of nonpolar fraction to the steam distillate from the deodorizer discharge.

In an attempt to increase the yield of Fraction I, SDC unsaponifiables were slurried with a small amount of pentane-hexane to a homogeneous paste and filtered. Washing the insolubles with cold pentane-hexane, followed by thorough drying under vacuum, isolated sterols of 97.1% purity. The overall fraction of the unsaponifiables produced 75% solubles and 25% insolubles (sterols). The solubles, when chromatographed (Fig. 5), indicated the expected decrease in sterols, accompanied by the desired increase in nonpolar Fraction I. The amount of sterols removed could be altered by a change in the amount of pentane-hexane used in slurrying although the overall increase in Fraction I probably would not be improved greatly. While readily producing quite pure

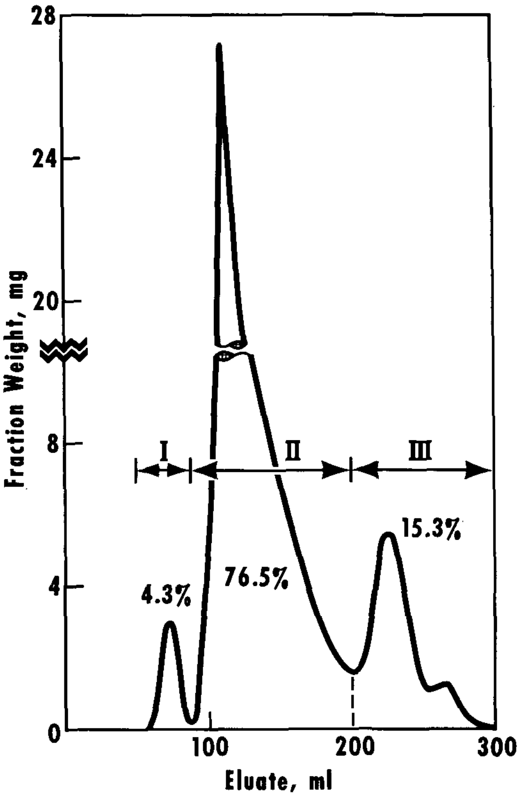

Fig. 4. Liquid-liquid partition chromatogram of saponified-acidified SDC.

sterols, this procedure only increased Fraction I by 3.8%, which was not sufficient for our purpose. Samples taken from the liquid chromatogram at peak I (hydrocarbon fraction) and at peak II (tocopherol) were analyzed for carbon and hydrogen; IR spectral curves were obtained also. In peak I the combined carbon-hydrogen was lower than expected for C_{30} hydrocarbons; and IR curves showed a slight hydroxyl absorption at 2.8 mμ, probably indicating some tocopherol contamination. In peak II the analyses agree well with the theoretical values given in Table I. Slight absorption occurred in the carbonyl region at 5.78 mμ in all samples.

Molecular distillation of whole SDC and 200C and 4 μ pressure provided volatile (20%) and nonvolatile (80%) materials from which unsaponifiables were extracted in yields of 80% and 18%, respectively. Figure 6 is a chromatogram of unsaponifiables obtained from the volatile material of molecular distillation, which provided a higher concentration of Fraction I than any other method. Unsaponifiables obtained in 18% yield from the nonvolatile fraction provided the chromatogram of Figure 7, showing a preponderance of sterols (89.8%) in Fraction III and indicating almost a complete removal of the volatile Fraction I by molecular distillation. Gas chromatograms of the various unsaponifiable fractions at 240C on a 6-ft 1% silicone rubber column indicated typical soybean unsaponifiables similar to chromatograms published previously (4).

TABLE I

Analysis of Nonpolar Fractions of Unsaponifiables from Shell Drain Condensate

Sample	Composition		
	Carbon %	Hydrogen %	Oxygen by difference %
Hydrocarbons from:[a]			
Beaker 13	83.42	11.86	4.72
Beaker 14	85.45	12.25	2.30
$C_{30}H_{50}$ (Squalene)	87.73	12.27	......
$C_{30}H_{62}$ (Squalane)	85.20	14.80	......
Tocopherol:[b]			
Beaker 13	80.65	11.59	7.76
Beaker 14	80.77	11.74	7.49
Theoretical	80.87	11.70	7.43

[a] Peak I samples from liquid chromatogram (Fig. 5).
[b] Peak II samples from liquid chromatogram (Fig. 5).

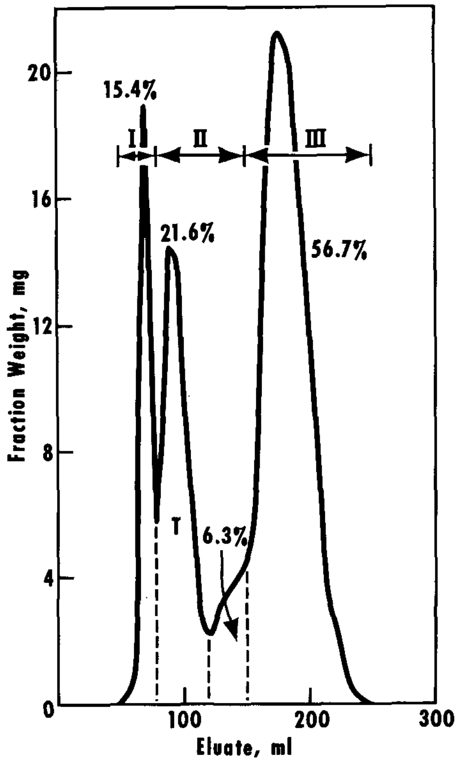

Fig. 5. Liquid-liquid partition chromatogram of pentane-hexane soluble portion of SDC unsaponifiables.

To separate the nonpolar hydrocarbon fraction from the tocopherol and sterol fractions, the acetylation-crystallization process of Baxter et al. (1) was employed. SDC unsaponifiables were acetylated by refluxing in acetic anhydride for 3 hr followed by cooling and hydrolysis of the excess anhydride with water. The acetic acid-water layer was extracted 10 times with diethyl ether and the ether extract finally washed 10 times with water to remove any dissolved acetic acid. The extract was thoroughly dried with anhydrous sodium sulfate, filtered, and evaporated to dryness under a slow stream of dry nitrogen. The dried residue, taken up in hot methanol, was crystallized by adding a small amount of water. Mother liquors from recrystallization of the acetates were combined before the solvent was removed. TLC analysis of the components of low volatility from the mother liquors indicated the presence of primarily nonpolar materials and the absence of free tocopherol.

Discussion

SDC from a soybean oil deodorizer was examined by chromatographic fractionation on silicic acid, and composition of each fraction was determined. Preliminary separation obtained by molecular distillation showed that the volatile portion contained a very high proportion (87%) of nonpolar material, including some neutral oil, sterol esters, hydrocarbons, and tocopherols. The free sterol content was reduced ca. 6.0% in the volatile fraction plus another 9.4% as sterol esters.

The high content of neutral oil and the essential

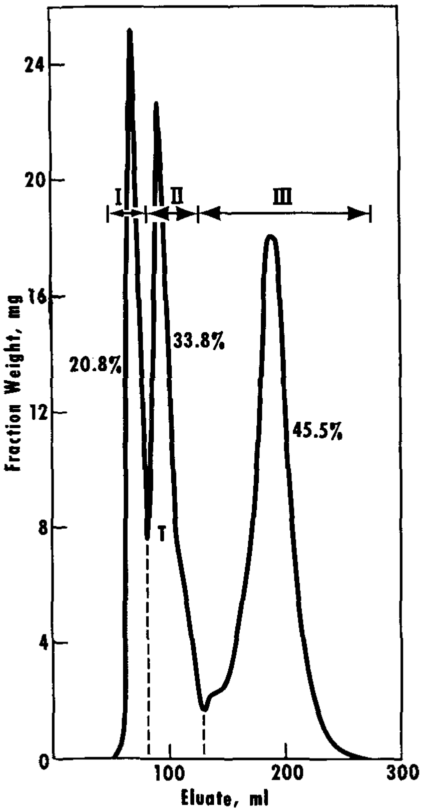

FIG. 6. Liquid-liquid partition chromatogram of unsaponifiables from volatile portion (200C at 4 μ) of molecularly distilled SDC.

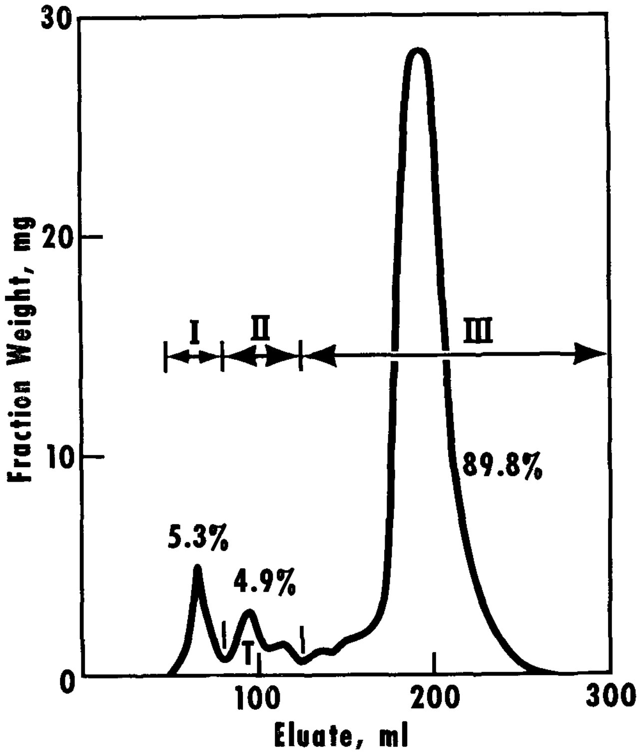

FIG. 7. Liquid-liquid partition chromatogram of unsaponifiables from the nonvolatile portion (200C at 4 μ) of molecularly distilled SDC.

absence of free fatty acids indicate a considerable difference in composition of the SDC and the hot-well skimmings of the deodorizer.

According to Fiala (2), SDC constitutes less than 10% of the loss in a deodorizer. Total deodorizer loss is reported at 0.1–0.3% by Jasperson and Jones (5) for vegetable oils and 0.4–0.6% by Fiala (2) for soybean oil. The composition of SDC is not recorded in the literature, and it is expected that the temperature of operation, design of deodorizer, as well as the kind and quality of the oil, markedly affect the composition of the various deodorizer distillates.

Sterols constitute over 70% of the unsaponifiables in SDC, which is more than double the percentage found in whole oil unsaponifiables (4). The remaining constituents are about equally divided between the tocopherols and the less polar hydrocarbons. Since the unsaponifiable content is 23% of the total SDC, a high concentration of nonpolar materials is available. Suitable distillation, liquid extraction, or large column chromatography should make quantities of these concentrates available in high purity.

Liquid fractionation as practiced for the preparation of pure sterols (7) showed that it was comparatively easy to obtain a relatively pure sterol fraction from the SDC unsaponifiables, but such a method did not appear promising as a means of obtaining a purified hydrocarbon fraction. The high concentration of neutral oil and the absence of free fatty acids in the SDC should make it possible to obtain a hydrocarbon concentrate through distillation techniques.

Falling film distillation at 200C and 4 μ showed almost a complete removal of the hydrocarbon-tocopherol fraction from the nonvolatile portion. Baxter et al. (1) used a similar distillation technique to obtain tocopherol concentrates from cottonseed oil and wheat germ oil. The entire hydrocarbon fraction was thus concentrated in ¼ the volume by a single pass through a falling film still. Further concentrations of the hydrocarbons by repeated molecular distillation under more selective conditions were not attempted. Previous work with GLC showed a wide overlapping in retention times of the sterols and hydrocarbons (4); GLC analysis also showed that the volatile fraction obtained by molecular distillation of SDC to be similar to the unsaponifiables obtained from the whole soybean oil.

Removal of the tocopherols from the nonpolar fraction through acetylation and crystallization offers a means of concentrating the natural hydrocarbons in soybean oil. The acetates of the tocopherols are readily attained. TLC examination of the reaction products and crystallizing mixtures indicates a complete esterification of the tocopherols present. Baxter et al. (1), however, report difficulties in obtaining crystallization of the esterified tocopherol isomers. Purification and characterization of the natural hydrocarbons occurring in soybean oil will be considered in future publications.

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REFERENCES

1. Baxter, J. G., C. D. Robeson, J. D. Taylor, and R. W. Lehman, J. Am. Chem. Soc. *65*, 918–924 (1943).
2. Fiala, R. J., JAOCS *36*, 375–379 (1959).
3. Frankel, E. N., C. D. Evans, Helen A. Moser, D. G. McConnell, and J. C. Cowan, *Ibid.*, *38*, 130–134 (1961).
4. Hoffmann, R. L., Helen A. Moser, C. D. Evans, and J C. Cowan, *Ibid.*, *39*, 323–327 (1962).
5. Jasperson, H., and R. Jones, J. Soc. Chem. Ind. *66*, 13–17 (1947).
6. Lange, W. J., JAOCS *27*, 414–422 (1950).
7. Poulos, A., J. W. Greiner, and G. A. Fevig, Ind. Eng. Chem. *53*, 949–962 (1961).
8. Scholfield, C. R., and W. C. Bull, Oil & Soap *21*, 87–89 (1944).

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