oil or corn oil impart a rancid and painty flavor to the oil but do not make it taste like reverted soybean oil.

The flavor compounds isolated by the present technique are characteristic of each oil. The expert flavor panel identified a bland coconut oil containing the volatile components isolated from one-week-old corn oil as corn oil.

During the present process for the isolation of flavor compounds, the oil is only heated to 80°C. for 12 min. Therefore no significant amount of flavor components is produced during the process, and the flavor isolated represents that originally present in the oil. This is further evidenced by two observations. One, when 5 gal. of freshly deodorized soybean oil were treated by the present process, no detectable amount of flavor compounds was obtained. Two, the decrease of peroxide number of the oil during the present process is less than one meq. per kg. The reverted soybean oils subjected to the present process had peroxide numbers ranging from 4.1–7.3 meq. per kg.; after passing through the column, the peroxide numbers were 3.4–6.6, respectively.

Due to the low temperature used in this process, the oil is not completely deodorized. Reverted soybean oil, after passing through the Oldershaw column of the present process, still has a reversion flavor but it is less intense.

The flavor compounds isolated by the present process are free from entrainment of oils. One-tenth ml. of the concentrated ethyl ether solution of reversion flavor of soybean oil as obtained by the present technique was spread on a rock salt plate. The plate was set in a vacuum desiccator at room temperature for 4 min. The residue left on the rock salt plate as a thin film had a strong typical reversion odor. After the plate was kept under vacuum overnight at room temperature, all the reversion odor disappeared and there was no oily residue left on the rock salt plate.

It was found that a 10-plate Oldershaw column can be used to replace the 30-plate column without seriously affecting the isolation of flavor compounds. When the short column is used, the steam stripping can be operated under a vacuum of 10 mm. Hg at the bottom of the column. It was also found that carbon dioxide can be used to replace steam. A high purity carbon dioxide gas cylinder was attached to the flowmeter K (Fig. 1). The carbon dioxide gas, after passing through the Oldershaw column, was condensed in the liquid nitrogen trap B as solid carbon dioxide. After the stripping operation the traps A and B were taken off and the liquid nitrogen in trap B was removed. The solid carbon dioxide condensed on trap B was allowed to evaporate slowly and the gas was led to pass through trap A, still cooled with dry ice. After all the solid carbon dioxide in trap B was evaporated, the flavor compounds left in both traps A and B were rinsed out with ethyl ether. This process is more time-consuming and is less efficient than using steam.

Acknowledgment

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REFERENCES

1. Martin, C., Schepartz, A. I., and Daubert, B. F., J. Am. Oil Chemists' Soc., *25*, 113–117 (1948).
2. Kawahara, F. K., and Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 372–377 (1952).
3. Bruyn, J. de, and Schogt, J. C. M., J. Am. Oil Chemists' Soc., *38*, 40–44 (1961).
4. Collins, F. C., and Lantz, Vernon, Ind. Eng. Chem., *18*, 673–677 (1946).

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Characterization of the Reversion Flavor of Soybean Oil

S. S. CHANG,[1] K. M. BROBST, H. TAI, and C. E. IRELAND,
A. E. Staley Manufacturing Company, Decatur, Illinois

The reversion flavor isolated from reverted-but-not-rancid soybean oil was separated into 14 fractions by gas-liquid chromatography. The gas chromatographic fractions were collected in specially designed gas cells and polyethylene capillary traps for the determination of their infrared spectra by micro techniques. Ethyl formate, ethyl acetate, ethyl alcohol, n-butyraldehyde, 2-heptanone, and 2-heptenal were positively identified. Presence of alcohol, ester, and possibly dimethyl amino compounds in the fractions with higher chromatographic retention times was indicated by infrared analyses. Ultimate analyses of the flavor compounds isolated from reverted-but-not-rancid soybean oil also indicated the presence of nitrogen compounds.

THE DEVELOPMENT of a characteristic beany and grassy flavor known as reversion during the storage of refined and deodorized soybean oil is a classical problem of the soybean oil industry. A number of mechanisms have been postulated for the formation of reversion compounds. None of them has been unequivocally accepted. Mattil (1) found that addition of the nonsaponifiable extract of hydrogenated soybean oil to either refined cottonseed oil or refined peanut oil caused these oils to develop flavors characteristic of reverted soybean oil. Chang and Kummerow (2) reported that oxidative polymers of polyunsaturated fatty esters, if formed during the processing of soybean oil, may serve as one of the precursors of the reversion compounds. This theory appears to have been substantiated by Evans *et al.* (3), who prepared oxidative polymers by heating peroxides of soybean oil to conditions approximating those of deodorization and found that addition of the dimers to soybean oil significantly decreases its flavor stability. But the most extensively studied hypothesis (4, 5, 6) is that the reversion compounds are oxidative decomposition products of linolenic acid. Recently 3-hexenal was reported to have a green bean odor (7). This seems to indicate that linolenic acid is at least one of the precursors of the reversion flavor of soybean oil.

On the other hand, there are a number of experimental observations which cannot be explained by the linolenic acid theory. The development of reversion

[1] Present address: Department of Food Science, Rutgers, The State University, New Brunswick, N. J.

flavor is noted even when soybean oil has been highly hydrogenated. Sims (8) hydrogenated soybean oil to linolenic acid contents from 8 to 0% and found no correlation between flavor stability and content of polyunsaturated triglycerides. Furthermore Robinson and Black (9) observed flavor deterioration in soybean oil aged under inert gases, and Bickford (10) reported that reversion could occur during storage of soybean oil under high vacuum.

Soybean oil therefore may have two problems of flavor stability. One is its poor stability in regard to the development of rancidity. The other is the development of reversion flavor when the peroxide number of the soybean oil is still low (1–3 meq./kg.). There is no doubt whatsoever that the presence of odor (7). This seems to indicate that linolenic acid and accelerates the development of rancidity (11). But whether linolenic acid is also completely responsible for the development of the characteristic beany and grassy flavor in soybean oil is still difficult to ascertain.

Recently salad oils were prepared by the winterization of selectively hydrogenated soybean oil. The linolenic acid contents of these oils were decreased by hydrogenation to as low as 0.5%. These oils are as good as cottonseed oil so far as oxidative and thermal stabilities as measured by AOM hours, increase in viscosity during deep-fat frying, and Schaal oven test of potato chips fried in these oils are concerned. However, when aged in a clear glass bottle under diffused daylight at room temperature, these salad oils and the potato chips fried in them again developed the characteristic reversion flavor. Jacobson (12) found good correlation between the content of carbonyl compounds in beef fat and its organoleptic flavor scores. But with soybean oil he found no correlation during the initial stage of reversion, and a fair correlation was observed during the later stages of aging. This may indicate that carbonyl compounds are not entirely responsible for the reversion flavor.

In order to understand the mechanisms involved in the development of reversion flavor, attempts were made by previous investigators to identify chemically the volatile decomposition products obtained by bubbling air into soybean oil until it was highly rancid and polymerized (5) or by repeated deodorization at 200°C. (13). The volatile decomposition products obtained by these processes contain relatively large amounts of oxidative and thermal decomposition products which may overshadow the extremely small amount of compounds which are truly responsible for the reversion flavor.

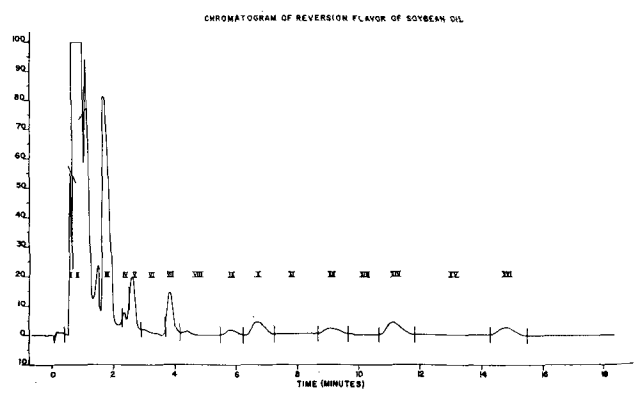

FIG. 1. Gas chromatogram of reversion flavor at 110°C. with Carbowax 20 M. as the stationary phase.

Recently a new technique has been developed which can isolate the characteristic reversion flavor from reverted-but-not-rancid soybean oil without creating decomposition products during the isolation process (14). The present paper reports the fractionation of this reversion flavor by gas-liquid chromatography and the chemical identification of the chromatographic fractions by micro-infrared spectrometry.

Experimental

Isolation of Reversion Flavor. Soybean oil refined with acetic anhydride and deodorized at 204°C. was used for this experiment. The oil was aged for two weeks at room temperature in completely filled glass jugs. The reversion flavor was then isolated according to the technique described by Chang (14). Approximately 4 ml. of ethyl ether solution containing approximately 0.4 g. of flavor compounds were obtained from 20 gal. of reverted-but-not-rancid soybean oil.

Gas Chromatography of Reversion Flavor. A model k-2 Burrel Gas Chromatograph with a thermoconductivity detector was used. The 2.5-meter-long column of 5 mm. in diameter was packed with 20% Carbowax 20 M. on Chromosorb. The column was conditioned by heating to 120°C. over-night under a current of helium. Before fractionating the flavor compounds, a cold trap was attached to the effluent gas from the column for at least 1 hr. to ensure that no appreciable amount of packing material was bleeding from the column. During the chromatography the rate of helium flow was maintained at 50 ml. per min.

Collection of Gas Chromatographic Fractions. A manifold of 18 outlets was made from stopcocks with 1-mm. bore of plug and capillary stems which have approximately the same bore as the hole in the plug. The manifold was wrapped with heating tape and kept at the same temperature as the chromatograph column to prevent condensation of the chromatographic fractions. A gas cell (15) or polyethylene capillary trap (16) was connected to each outlet and cooled with powdered solid carbon dioxide. The manifold was connected to the exit of the gas chromatograph. By manipulating the stopcocks, the effluent gas can be directed into one of the 18 traps, in each of which one chromatographic fraction was condensed. The time interval of the gas current flowing from the detector cell to the cold trap was measured as 10 seconds. This delay was allowed in manipulating the stopcocks.

Four microliters of the ethyl ether solution were chromatographed at 110°C. Each chromatographic fraction, as indicated on the chromatogram (Figure 1), was collected in one cold trap. This was repeated 42 times. Each chromatographic fraction was accumulated in one cold trap.

The third fraction contains more than one peak. It was rechromatographed in the same manner at 70°C. Each chromatographic fraction, as indicated on the chromatogram (Figure 2), was collected in one cold trap.

All the fractions were rechromatographed once more to produce well-defined peaks before they were subjected to analyses.

Determination of Infrared Spectrum. The infrared spectrum of each of the chromatographic fractions was determined in gas cell, in cavity cell, and/or in polyethylene capillary, depending upon its volatility and the amount available. A Beckman IR-4 Spectro-

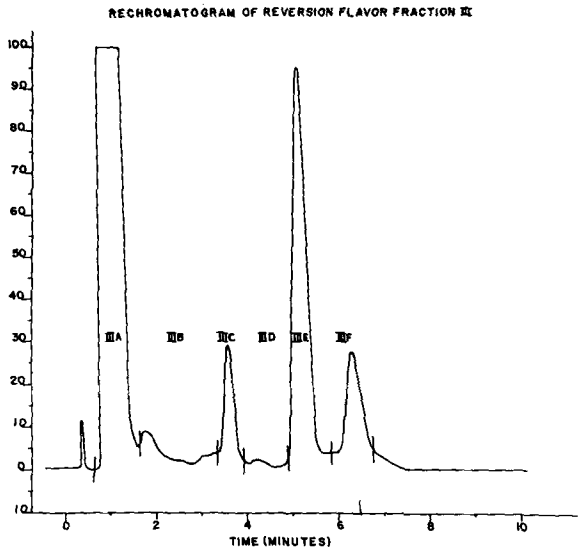

FIG. 2. Gas chromatogram of Fraction III at 70°C. with Carbowax 20 M. as the stationary phase.

TABLE I

Identification of Gas Chromatographic Fractions

I. Fractions identified	
III C	Ethyl formate
III E	Ethyl acetate
III F	Ethyl alcohol
VII	n-Butyraldehyde
XII	2-Heptanone
XIV	2-Heptenal
II. Fractions which are solvent	
II	
III A	
III. Fractions not obtained in sufficient amount for infrared spectra	
III B	
III D	
IV	
V	
IV. Fractions not identified	
VII	Aldehyde and ester
IX	Ketone
X	Alcohol
XVI	Ester

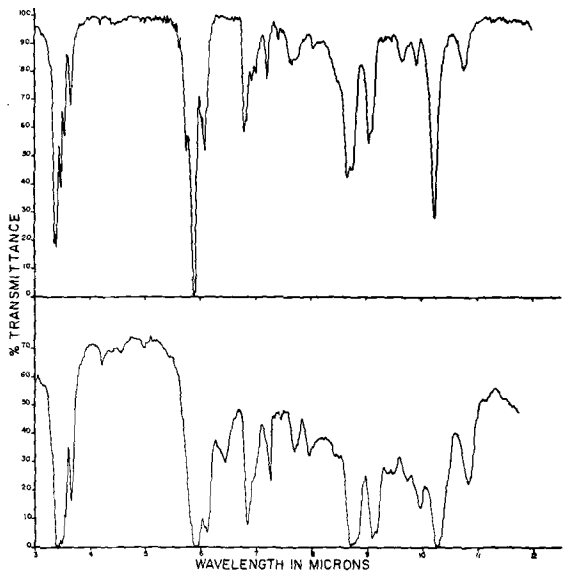

FIG. 3. Infrared spectra of 2-heptenal (top curve) and Fraction XIV (bottom curve) as measured in rock salt cavity cell with carbon tetrachloride as solvent.

photometer with a beam condenser attachment was used. When solvent was used in the determination of the infrared spectrum, a variable thickness cell was used in the reference beam to compensate for the absorption of the solvent.

Identification of Infrared Spectrum. Wyandotte-A.S.T.M. Punched Cards for infrared spectrum and the Sadtler Spectrum with Spec Finder were used as reference spectra for the identification of gas chromatographic fractions.

Results and Discussion

Gas chromatography of the reversion flavor isolated from reverted-but-not-rancid soybean oil yielded 14 fractions, each corresponding to a distinct peak on the chromatogram (Figures 1 and 2). Each of the chromatographic fractions has its characteristic odor, some of which are definitely not associated with aldehydes and ketones.

Six fractions have been chemically identified by their infrared spectra (Table I). They are ethyl formate, ethyl acetate, ethyl alcohol, n-butyraldehyde, 2-heptanone, and 2-heptenal. These fractions have infrared spectra identical with those of their respective reference spectra, as illustrated with 2-heptenal (Figure 3).

Four fractions, IIIB, IIID, IV, and V, were not obtained in sufficient amount for well-defined infrared spectra. Fractions II and IIIA are ethyl ether (Table I).

Fraction V is of great interest because it has an extremely strong and highly characteristic buttery odor. Daubert (17) described the first flavor developed from a bland, freshly deodorized soybean oil as "buttery" or "mild beany." Buttery is also a descriptive term commonly used in the organoleptic evaluation of oils. Now a buttery odor is isolated and detected as a gas chromatographic peak. It is also

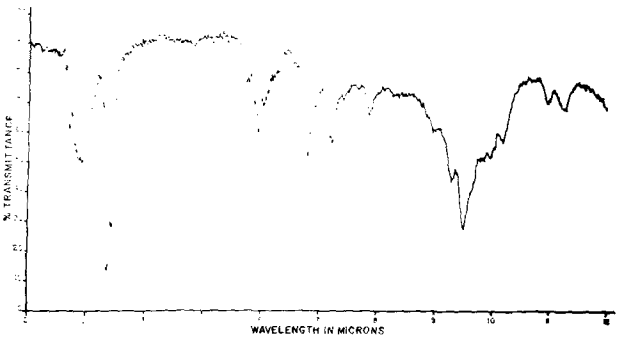

FIG. 4. Infrared spectrum of Fraction X as measured in rock salt cavity cell with carbon tetrachloride as solvent.

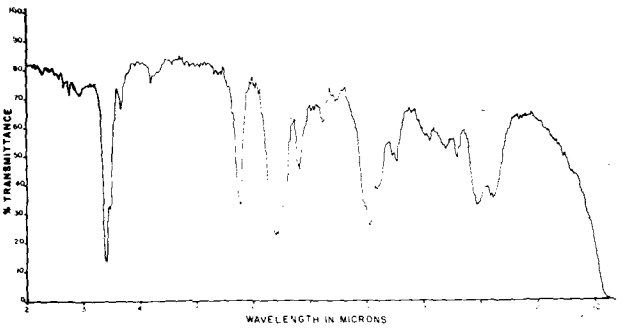

FIG. 5. Infrared spectra of Fraction XVI as measured in rock salt cavity cell with carbon tetrachloride as solvent.

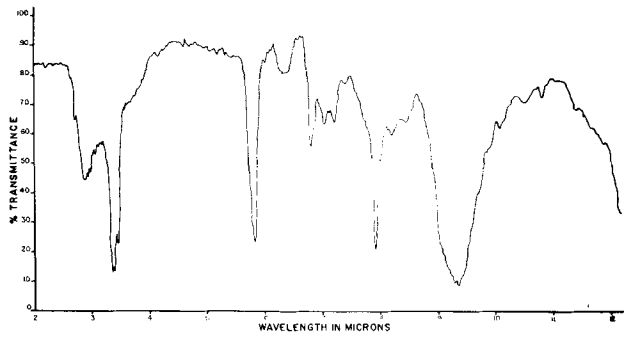

FIG. 6. Infrared spectrum of Fraction A as measured in cavity cell with carbon tetrachloride as solvent.

important to note that this buttery fraction is developed during the initial stage of reversion. When the soybean oil is highly reverted, this small buttery fraction tends to be overshadowed by the relatively large amounts of other flavor compounds.

Well-defined infrared spectra of the remaining four fractions were obtained. But their identifications are not complete.

Fraction VIII may be impure. Its infrared spectra indicates two carbonyl groups, one ester and the other aldehyde. Fraction IX is a ketone, probably a homologue of 2-heptanone. Fraction X (Figure 4) is an alcohol. Fraction XVI (Figure 5) is an ester.

An additional peak A was obtained from a repeat run of the reversion flavor. The infrared spectrum of this fraction (Figure 6) has a strong band at 5.8 μ and is therefore a carbonyl compound. It also has bands at 7.9 and 9.3 μ which are difficult to interpret. In the reference spectra only dimethyl formamide has an arrangement similar to those of these peaks.

The possibility of the presence of nitrogen compounds in reversion flavor is enhanced by ultimate analyses. The reversion flavor isolated from soybean oil which has been refined with acetic anhydride contains 0.67% of nitrogen. One of the possible precursors of nitrogen compounds is phosphatide. Soybean phosphatides were purified by precipitation from acetone and prepared in granular form. Flavor compounds isolated from the soybean phosphatides at room temperature contain 2.72% nitrogen. Therefore if phosphatides are not completely removed from the oil during refining, they may serve as one of the precursors of nitrogen compounds.

REFERENCES

1. Mattil, K. F., J. Am. Oil Chemists' Soc., *24*, 243–246 (1947).
2. Chang, S. S., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *31*, 324–327 (1954).
3. Evans, C. D., Frankel, E. N., Cooney, P. M., and Moser, H. A., J. Am. Oil Chemists' Soc., *37*, 452–456 (1960).
4. Dutton, H. J., Lancaster, Catherine R., Evans, C. D., and Cowan, J. C., J. Am. Oil Chemists' Soc., *28*, 115–118 (1951).
5. Kawahara, F. K., and Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 372–377 (1952).
6. Dutton, H. J., Evans, C. D., and Cowan, J. C., Trans. Am. Assoc. Cereal Chemists, *11*, 116–135 (1953).
7. Hoffmann, G., J. Am. Oil Chemists' Soc., *38*, 1–3 (1961).
8. Sims, R. J., J. Am. Oil Chemists' Soc., *29*, 347–350 (1952).
9. Robinson, H. E., and Black, H. C., Ind. Eng. Chem., *37*, 217–219 (1945).
10. Bickford, W. G., Oil and Soap, *18*, 195 (1941).
11. Kartha, A. R. S., J. Sci. Ind. Res. (India), *17B*, 135 (1958).
12. Jacobson, G. A., Proceedings of Flavor Chemistry Symposium, Camden, N. J., 1961, Campbell Soup Company, pp. 165–178.
13. Martin, C., Schepartz, A. I., and Daubert, B. F., J. Am. Oil Chemists' Soc., *25*, 113–117 (1948).
14. Chang, S. S., J. Am. Oil Chemists' Soc., *38*, 669–671 (1961).
15. Chang, S. S., Ireland, C. E., and Tai, H., Anal. Chem., *33*, 479 (1961).
16. Chang, S. S., Brobst, K. M., Ireland, C. E., and Tai, H., J. Applied Spec., in press.
17. Daubert, B. F., and O'Connell, P. W., "Advances in Food Research," Vol. IV, Academic Press Inc., 1953, pp. 185–207.

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Correlation of the Mean-Molecular Weights of Commercial Alkylbenzenes with Gas-Liquid Chromatographic Data

A. H. SILVER, W. H. ADAM, H. M. GARDNER and H. J. KEILY, Lever Brothers Company, Research and Development Division, Edgewater, New Jersey

Gas-liquid chromatography has been used to estimate the mean-molecular weights of commercial alkylbenzenes of the polypropylene type. Results obtained chromatographically on samples from several sources correlate well with mean-molecular weight data determined chemically via the sulfonic acids produced from them.

The chromatograms are obtained with a 200-ft. capillary column coated with Apiezon L grease. Partial resolution of the multicomponent mixtures to yield eighty to one hundred peaks, each possibly representing several components, is achieved. A plot of the logarithms of the relative retention times at the median areas of the chromatograms of narrow-cut distillation fractions of alkylbenzene vs. their known mean-molecular weights provides a linear calibrating relationship with a discontinuity at a mean-molecular weight of ca. 260. This discontinuity distinguishes "dodecylbenzenes" from the higher molecular weight "tetradecylbenzenes."

MIXTURES OF phenyl-n-dodecanes and didodecylbenzenes have been analyzed by gas-liquid chromatography using capillary columns in conjunction with an argon ionization detector (1). This separation technique has also been applied to the characterization of commercial alkylbenzenes of the polypropylene type (2). As an extension of the latter application, the work presented in this paper describes a method for evaluating the partially resolved chromatograms of commercial alkylbenzenes in terms of their mean-molecular weights.

When the chromatograms of narrow-cut distillation fractions of alkylbenzene of the polypropylene type are examined, it is found that the relative retention time at the median area of each chromatogram is shifted exponentially to higher values with a linear