Studies on the Flavor of Autoxidized Soybean Oil[1]

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Abstract

The flavor components of soybean oil in the early stages of autoxidation were isolated by distillation in a molecular still. The distillate consisted of an aqueous layer and an oily film. The oily film did not reproduce the autoxidized flavor when added to freshly deodorized oil. Gas chromatographic and organoleptic analysis indicated that the oily film contained hexanal, vinylamyl ketone, and *trans,cis*-2,6-nonadienal. The aqueous layer reproduced the autoxidized flavor when added to freshly deodorized oil, and the flavor had a retention time on butandiol succinate columns between those of pentanal and hexanal. Mixtures of vinylethyl ketone and pentanal gave a flavor to freshly deodorized oil similar to the flavor of oil in the early stages of autoxidation. Vinylethyl ketone was identified in the distillate from autoxidized soybean oil as the 2,4-dinitrophenylhydrazone.

Introduction

IT IS GENERALLY AGREED that the compounds responsible for the flavors of autoxidized fats are aldehydes and ketones, and a great deal of work has been done identifying the carbonyl compounds that are produced (8). In most tests, the compounds isolated have not satisfactorily reproduced the flavor of the autoxidized fat from which they were isolated, and it has been suggested that the flavors are caused by a large number of carbonyl compounds blended in just the right proportions (4). Work in our laboratory with autoxidized milk fat has indicated that most of the carbonyl compounds produced during autoxidation have very little role in producing flavor sensations. The dominant flavor characteristics of autoxidized milk fat can be reproduced by three compounds: vinylamyl ketone, 2,4-decadienal, and *trans,-cis*-2,6-nonadienal (6,7). The first two of these compounds arise from linoleate and the third from linolenate. Since linoleate and linolenate are important constituents of many vegetable oils, we decided to see if these same compounds could account for the flavor of autoxidized soybean oil.

Methods

The soybean oil was obtained from Swift and Company and had been refined by their usual procedures and stabilized with 0.005% citric acid. This oil was redeodorized in small batches as needed (10). When freshly deodorized, the soybean oil was bland and had a slight nut-like flavor. When allowed to autoxidize at room temperature in a darkened bottle, the oil first developed a somewhat butter-like flavor. On further autoxidation, the oil developed a characteristic flavor which we term soybean oxidation flavor "A". On still further autoxidation, the flavor became more like that of autoxidized corn or cottonseed oil, and we call this flavor rancid. The time needed to develop flavor "A" varied from overnight to two weeks depending on the condition of the oil before redeodorization in the laboratory. The flavor compounds were isolated from oil in flavor stage "A" by slowly passing it through a Rota-Film still at or above room temperature (5). The flavor components were collected in a liquid nitrogen-cooled trap. The trap contents consisted of an aqueous layer and an oily film. The oily film was extracted with a small amount of petroleum ether. Both the aqueous layer and oily film were subjected to gas chromatography in a locally constructed gas chromatograph with a thermal conductivity detector. The columns were 6 ft long and 0.375 or 0.25 in. in diameter and were filled with 30% butanediol succinate on Chromosorb P. The carrier gas was helium. The oily layer was examined at 140C; the aqueous layer at 125C. The two layers were also analyzed in an Aerograph Hi-Fy instrument with a flame ionization detector. Here, the column was 0.125 in. Teflon tubing packed with 30% butanediol succinate on Chromosorb P. The temperature was 154C.

Cottonseed, corn, and soybean oil were deodorized for the organoleptic evaluations (10). The fractions from the gas chromatograph were collected by bubbling the tail pipe gas into the freshly deodorized oil, and the oil was tasted by a trained panel.

2,4-Dinitrophenylhydrazones of the carbonyl compounds in the distillate were prepared as an aid in identification. It is necessary to avoid strongly acidic conditions and polar solvents to obtain satisfactory derivatives of the vinyl ketones (11). To accomplish this, flavor distillate was mixed with enough sodium sulfate to remove the water. Then, a solution of 2,4-dinitrophenylhydrazine in tetrahydrofuran-acetic acid (4:1) was added. The tetrahydrofuran was freshly distilled over sodium. The reaction mixture was left at room temperature overnight, the solvent was removed under reduced pressure, and the 2,4-dinitrophenylhydrazones were extracted with Skellysolve B. The 2,4-dinitrophenylhydrazones were fractionated by thin-layer, partition paper chromatography and partition column chromatography. Thin-layer chromatography was carried out on Silica gel G (7) and Silica gel G-silver nitrate (1) using petroleum ether with 4 to 7% diethyl ether as the mobile phase. The paper chromatography was carried out on kerosene impregnated paper, with methanolethanol water, 10:10:5, as the mobile phase (5). Partition column chromatography was carried out on nitromethane-Skellysolve B columns (3).

Results and Discussion

Distillation of the Flavor

The flavor of soybean oil in the early stages of autoxidation that we call soybean flavor "A", was not completely removed by passing the oil through the Rota-film still at room temperature. In previous work, we found autoxidized milk fat and trilinolein to be effectively deodorized by passage through the still, but the beany flavor of autoxidized trilinolenin was not removed (6). Distillation at higher temperatures did not improve the flavor of the soybean oil. At 70C, the residual oil still had some flavor A and a slightly fishy flavor, and, at 170C, a fishy and "heated oil" flavor. Although no manipulation of the

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conditions gave a bland oil, it was subsequently found that the flavor components isolated from the oil are relatively volatile, especially those responsible for flavor A. Evidently precursors are present in the oil which can generate the flavor A rapidly under vacuum. These may be polymers formed during autoxidation as proposed by Chang and Kummerow (2).

Gas Chromatography and Organoleptic Studies

When the aqueous and oily layers of the flavor distillate were incorporated into freshly deodorized oil, the aqueous layer gave a faithful reproduction of flavor A, but the oily film did not give flavor A.

The aqueous layer was fractionated by gas chromatography using a thermal conductivity detector. Water was adsorbed on the chromatography packing and emerged from the column in a peak that trailed out for at least 30 min, so the peaks of other components were obscured. However, fractions from the gas chromatograph could be taken at various time intervals and tasted in freshly deodorized oil. A series of n-aldehydes were separated on the gas chromatograph under the same conditions of flow and temperature as the aqueous layer. The retention times of the aldehydes and their peak widths were used as a guide in selecting the time intervals for taking the flavor samples during the chromatography of the aqueous layer. The presence of water vapor seems to have no effect on the retention time of the components. This was demonstrated by the almost identical retention times of common peaks of the aqueous layer and oily film when a hydrogen flame detector, which does not respond to water vapor, was used. This procedure revealed that flavor A was reproduced by a component having a retention time between those of pentanal and hexanal. No other flavors were found in the fractions from the aqueous layer.

The oily film was also fractionated by gas chromatography and contained three distinguishable flavors. The first and most intense was a flavor suggestive of a green plant. It had the same retention time as hexanal, and the flavor could be reproduced by pure hexanal. The second flavor was oxidized-metallic and had a retention time like octanal. It was probably vinylamyl ketone. The third flavor had a retention time close to that of undecanal and had a straw-like, grassy flavor. It was probably *trans,cis*-2,6-nonadienal. These flavors could be detected only by tasting them in considerably less oil than that from which they were isolated, so it is doubtful that they make a significant contribution to soybean flavor A.

The aqueous layer and oily film were also fractionated by gas chromatography using a hydrogen flame detector, and the retention time of the components that were detected are compared with those of standard aldehydes in Table I. The two layers had similar compositions qualitatively. The component with a retention time of 0.9 min in the aqueous layer may be responsible for flavor A. The component with a retention time of 1.1 in both the aqueous layer and oily film is probably hexanal, and this component is responsible for the green plant flavor in the oily film. No peaks were detected which can be correlated with the oxidized-metallic and grassy flavors of the oily film, but this is not unexpected since the organoleptic test for these compounds is considerably more sensitive than the hydrogen flame detector.

TABLE I

Retention Time of Compounds in the Aqueous and Oily Layer of Autoxidized Soybean Oil Distillate Compared with Standard Aldehydes

Oily film [a] min	Aqueous layer [a] min		Aldehydes min
	0.9	C_5	0.8
1.1	1.1	C_6	1.1
1.2			
1.3 [b]		C_7	1.5
2.8		C_8	2.2
3.1	3.1 [c]	C_9	3.2
4.0 [b]	4.0 [c]		
4.6	4.7 [b]	C_{16}	4.8
5.3 [b]	5.4		
9.3	8.8	C_{11}	7.2
	17.2 [c]		

[a] Peaks earlier than 0.9 obscured by solvent effect.

[b] Minor components.

[c] Major components.

The component responsible for soybean flavor A was quite unstable, and attempts to concentrate it from the aqueous layer by gas chromatography were not successful.

Hoffman isolated *cis*-3-hexenal from autoxidized soybean oil and reported that it was responsible for the "green beany" flavor which was "the first impression which was usually perceptible after deodorization . . ." (9). *Cis*-3-hexenal has a retention time slightly longer than hexanal when chromatographed on butanediol succinate. It had a green plant flavor quite similar to hexanal in soybean oil and did not account for soybean flavor A.

Vinylamyl ketone arises from the autoxidation of linoleate and has an intense flavor (7). By analogy, vinylethyl ketone, a five carbon ketone, should arise from the autoxidation of linolenate. Vinylethyl ketone like soybean flavor A has a retention time between that of pentanal and hexanal on butanediol succinate columns. It gives an intense flavor to soybean oil at 10 ppb, but the flavor does not resemble flavor A.

Vinylamyl ketone has a metallic flavor when tasted by itself in oil or milk, but, when it is mixed with small amounts of aldehyde, it gives an entirely different flavor — the typical oxidized flavor of diary products (7). Vinylethyl ketone behaves similarly. A mixture of vinylethyl ketone with approximately equal quantities of pentanal in oil gave a flavor similar to, but not identical with, the flavor A. The best results were obtained when both components were at about 10 ppb. If the amount of pentanal exceeded that of the vinylethyl ketone about 1.5 fold, the flavor intensity decreased and became less suggestive of flavor A. Blends of vinylethyl ketone with various combinations of hexanal, *cis*-3-hexenal, and pentanal did not reproduce flavor A any better than vinylethyl ketone and pentanal alone.

Isolation of Vinylethyl Ketone from Soybean Oil

The presence of vinylethyl ketone in soybean oil was demonstrated by isolation of its 2,4-dinitrophenylhydrazone. This was carried out as follows: The aqueous layer obtained from the molecular distillation of 3 liters of oil exhibiting soybean flavor A was reacted with 2,4-dinitrophenylhydrazine. The 2,4-dinitrophenylhydrazone were isolated and recrystallized from a small amount of petroleum ether to concentrate the minor constituents in the mother liquor. The 2,4-dinitrophenylhydrazones in the mother liquor were separated on thin-layer silica gel plates, and a small amount of material having the same R_f as vinylethyl ketone was recovered. This material was further purified by chromatography on kerosene impregnated paper and nitromethane-petroleum ether columns. A 2,4-dinitrophenylhydrazone isolated from the nitromethane column with an absorption maximum at 367 mμ, in agreement with that of the authentic derivative of vinylethyl ketone. The isolated material had the same R_f as the authentic derivative when chromatographed on silica gel-silver nitrate plates, or when rechromatographed on silica gel plates and kerosene impregnated paper.

This evidence suggests that vinylethyl ketone in combination with other carbonyl compounds produces flavor A typical of the early stages of soybean oil autoxidation, just as vinylamyl ketone in combination with any of several aldehydes produces the oxidized flavor typical of the early stages of autoxidation in milk fat. The importance of these vinyl ketones in the flavor is not due to their high concentrations relative to other carbonyl compounds, but rather to their extremely low flavor thresholds.

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Hydrogenation of Linolenate.

XII. Effect of Solvents on Selectivity[1]

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Abstract

Selectivity of heterogeneous catalysts for hydrogenation of linolenate over linoleate is increased by the presence of certain polar solvents. A ratio of specific reaction rate constants for linolenate to linoleate of 4 was obtained with a 5% palladium-on-alumina catalyst when dimethyl formamide (DMF) was used as the solvent. This high selectivity of DMF was independent of temperature and catalyst concentration. Other solvents that improved selectivity include furfural, acetonitrile, tetramethyl urea and trimethyl phosphate.

Introduction

THE CAUSE FOR CERTAIN undesirable flavors in soybean oil has been attributed to the presence of linolenic ester groups (1). A cooking or salad oil of increased stability can be obtained from soybean oil by a two-step process: a) selective hydrogenation, to reduce linolenate from its original 7–9% to 2–3%, and b) winterization, to remove the solid high-melting fatty acid esters formed during hydrogenation (2). Commercially available catalysts have selectivity ratios (^{K}linolenate/^{K}linoleate) of 1.8–2.9 (3,4). A ratio of 4 or greater, as calculated on an analog computer (5), is required to reduce the linolenate content in a liquid soybean oil to 2% or less without substantial loss of linoleate or increase in high melting glycerides, and such ratios increase the yield of liquid oil during winterization or may eliminate the need for that processing step.

When evaluating solvents for the selective extraction of linolenic acid-containing triglycerides from soybean oil, the possibility was suggested of hydrogenating in a two-phase oil-solvent system. By suspending a catalyst in the selective solvent layer, it was hoped to reduce preferentially the more unsaturated triglycerides and improve selectivity. As reduction proceeded, an equilibrium would be maintained between oil and solvent phases; thus during the hydrogenation the more unsaturated triglycerides from the oil phase would tend to move into the solvent layer and the more saturated triglycerides produced in the solvent phase would tend to migrate into the oil phase. Hydrogenating in the presence of solvents has been covered in Maryott's patent (6), and the concept of hydrogenating in a selective solvent phase has been disclosed in another (7). However, the purpose of the second patent was to reduce the polyunsaturated esters to monounsaturated esters without producing any saturated esters, whereas our study is concerned with increasing the hydrogenation rate of linolenate to linoleate.

Experimental

Materials

Solvents evaluated were of the purest grade commercially available and were used without further

TABLE I

Hydrogenation of Linseed: Safflower Oil Mixture
(Temp. 25C, atmospheric pressure, 0.06% of 5% Pd-on-alumina catalyst)

Sample	Time of hydrogenation min.	Composition, %			IV Calcd	*trans* %	Selectivitity (^{K}Le/^{K}Lo)
		01	Lo	Le			
Starting material	—	16.2	37.8	36.7	175.3	—	—
No solvent	70	34.6	41.2	14.2	138.3	14.4	1.85
5 Volumes DMF + 1 Volume oil	18	31.5	49.6	9.7	138.4	13.0	3.98

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[1] Presented at AOCS meeting in Chicago, 1964.

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