Tocopherol Concentrates by the Fractional Crystallization of Cottonseed Oil From Solvents[1]

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Introduction

Low temperature fractional crystallization from solvents has been under investigation in this laboratory for some time, as a tool for the fractionation of fatty materials to produce new, so-called "tailor-made" fats and oils. Certain data obtained in the course of this work indicated the possibility of employing the same technique to produce tocopherol concentrates from vegetable oils. At present, such concentrates are prepared almost exclusively by molecular distillation.

The method used in preparing the concentrates consisted of dissolving the fat in a solvent, and crystallizing out the bulk of the glycerides at a low temperature to leave a small residue of fatty material in the solvent. If the crystallization is properly carried out, this uncrystallized residue will contain most of the tocopherols in the fat, but only a very small portion of the glycerides. The present investigation was confined to refined cottonseed oil. Since this oil usually does not contain more than 0.05 to 0.10 percent tocopherols, it is necessary to reduce the uncrystallized residue to about 0.5 to 1.0 percent of the oil, in order to obtain residues or concentrates with tocopherol concentrates as high as 10 percent.

Hydrogenated oil was used in some of the experiments, since the experience of previous workers has indicated that crystallization of unhydrogenated vegetable oils at reasonably low temperatures was not likely to result in a sufficient removal of glycerides to produce residues high in tocopherol content. Thus, for example, Bull and Wheeler (1) found that the residue remaining from the crystallization of soybean oil from acetone at —50° to —70° C. could not be reduced below 2 to 3 percent. Riemenschneider, Swift, and Sando (5) reported that 4.8 percent of a sample of cottonseed oil remained soluble in acetone at —60° to —65° C.

Experimental Procedure

Two hundred grams of fat were dissolved in the proper amount of solvent. The solution was cooled by immersing the beaker containing it in a bath of acetone and dry ice. For temperatures lower than —78° C., liquid nitrogen was used in the chilling bath. After the desired low temperature was reached (usually in 30 to 45 minutes), the mixture of crystals and liquid was filtered by suction, through filter paper in a 20 cm. jacketed Büchner funnel. The funnel was cooled to the same temperature as the solvent and oil. The crystals were washed once on the filter with 75 ml. of solvent cooled to the temperature of the chilled crystals and liquid. Most of the solvent was removed from the filtrate by distillation; the last traces were swept out of the oily residue by a current of hydrogen at the temperature of a steam bath.

Tocopherol assays were made by the Parker and McFarlane modification (4) of the Emmerie-Engel method. Stability tests made to check the antioxygenic activity of the concentrates were carried out by aeration of the samples at 110°, according to Mehlenbacher's (3) modification of the Swift method.

The Samples of Oil Used

The cottonseed oil used in all the tests was obtained from a commercial source, and had been refined and bleached. Its iodine value was 103.2, and its tocopherol content, by the modified Emmerie-Engel method, was 0.05 percent, which is rather low for cottonseed oil. Portions of the oil were hydrogenated in the laboratory, one portion being partially hydrogenated to an iodine value of 59.5, and the other being almost completely hydrogenated to an iodine value of 0.44. These three oils, respectively, are referred to hereafter as unhydrogenated, partially hydrogenated, and completely hydrogenated. Each of the oils was steam deodorized at 400°F. for 30 minutes.

Nature of the Uncrystallized Residues

Detailed analyses of the concentrates were not made. However, in addition to containing tocopherols, the concentrates appeared to consist largely of glycerides. Analysis of one concentrate, which was obtained from an acetone solution of the partially hydrogenated oil (ratio, 16:1) by filtering, at —60° C., and evaporating solvent from the filtrate, revealed the presence of 82 percent saponifiable material. The tocopherol content of this sample was 9.4 percent. In every case the consistency of the concentrates was similar to that of the oil from which they were derived; i.e., the concentrates from the unhydrogenated oil were liquid, those from the partially hydrogenated oil were semi-solid, and those from the completely hydrogenated oil were hard and brittle.

Crystallization from Acetone at a Solvent-Oil Ratio of 8:1

In most of the experiments, acetone was used as the solvent in the proportion of 8 parts by weight to 1 part of oil. The essential data on a series of crystallizations carried out under these conditions at different temperatures are presented graphically in Figures 1 and 2. Figure 1 shows the yield of concentrate obtained at different temperatures, and Figure 2 shows the tocopherol content of the concentrates.

It is evident that high-tocopherol concentrates can be obtained from hydrogenated cottonseed oils but not from the unhydrogenated oil. The failure of the latter to yield concentrates high in tocopherol content is due to the relatively high solubility of the unhydrogenated glycerides. In the case of the partially hydrogenated oil, the solubility of the glycerides decreased

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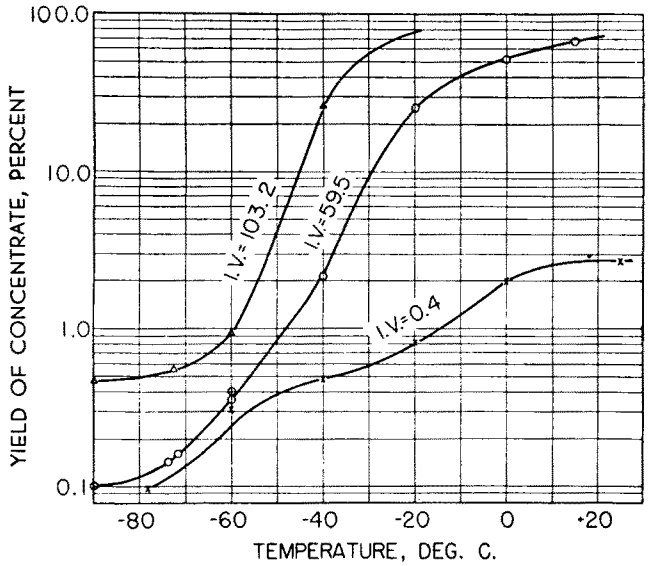

FIG. 1. Percent yield of concentrate from unhydrogenated, partially hydrogenated, and completely hydrogenated cottonseed oil at various crystallization temperatures. Acetone-solvent ratio 8:1 by weight.

rapidly as the temperature was lowered, with a corresponding increase in tocopherol content of the soluble portion, which rose to 37.8 percent at —90° C. However, concentrates from the unhydrogenated oil increased but slowly in tocopherol content as the temperature was decreased, and reached a maximum of 6.4 percent at —78° C.

At temperatures above —60° C., completely hydrogenated oil yields concentrates considerably higher in tocopherols than does partially hydrogenated oil, but at lower temperatures, there appears to be little if any advantage in hydrogenating the oil below an iodine value of about 60.

The recovery of tocopherols represented by each concentrate prepared, in terms of the amount of tocopherols originally present in the oil, is shown in Table 1, last column. The uncertainty involved in estimating the low tocopherol content of original oil makes it impossible to determine the recovery with high accuracy, but it is evident that most of the tocopherols appear in the concentrate if the temperature of crystallization is not below —74° C. A practicable operating temperature would appear to be about —72° C., at which temperature about 95 percent of the tocopherols are recovered, and a concentrate containing about 30 percent tocopherols is obtained.

At temperatures in the neighborhood of —70° C., concentrates can be obtained which are higher in tocopherol content than those obtainable by laboratory molecular distillation of the same oil. In a previous experiment (6), partially hydrogenated cottonseed oil subjected to a single molecular distillation yielded concentrates assaying no higher than 12.6 percent tocopherols, whereas solvent fractionation at —70° C. yielded a fraction containing about 27 percent tocopherols. In commercial practice, however, concentrates containing 27 percent or more of tocopherols are obtained by multiple molecular distillation in centrifugal stills (2).

Since the oil used in these experiments was relatively low in tocopherol content, it would appear

TABLE 1

Recovery of Tocopherols by Solvent Crystallization From Acetone (8:1 Ratio).

Oil	Temperature °C.	Yield of concentrate, gms. per 100 gms. oil	Tocopherol content of concentrate, percent	Recovery of tocopherols	
				Gms. per 100 gms. oil used	Percent recovery
Unhydrogenated	—60	0.94	5.40	0.051	102
Unhydrogenated	—90	0.47	4.1	0.019	38
Partially hydrogenated	0	52.58	0.097	0.051	102
Partially hydrogenated	—20	25.58	0.199	0.051	102
Partially hydrogenated	—60	0.40	12.4	0.050	100
Partially hydrogenated	—72	0.16	29.6	0.047	94
Partially hydrogenated	—74	0.14	32.1	0.045	90
Partially hydrogenated	—90	0.10	37.8	0.038	76
Completely hydrogenated	0	2.03	2.14	0.043	86
Completely hydrogenated	—40	0.48	10.3	0.049	98
Completely hydrogenated	—60	0.305	13.1	0.040	80

reasonable to expect that concentrates somewhat higher in tocopherols might be prepared from many cottonseed oils. The results are of course not directly applicable to oils other than cottonseed oil. However, if other oils were hydrogenated to an equivalent degree, they would not be expected to behave very differently from hydrogenated cottonseed oil. The tocopherol content of concentrates obtained from highly hydrogenated oils of different kinds should depend principally upon the tocopherol content of the original oil, and should be very nearly independent of the original glyceride composition of the oil.

Crystallization from Acetone at Other Solvent-Oil Ratios

Experiments were also made in which the partially hydrogenated oil was crystallizel at —60° from acetone, using solvent-oil ratios of 16:1 and 4:1 by weight. Data on these crystallizations, in comparison with a similar crystallization carried out with a solvent-oil ratio of 8:1, are listed in Table 2.

Crystallization at a solvent-oil ratio of 16:1 produced a concentrate lower in tocopherols than crystallization at a ratio of 8:1, and did not result in any material improvement in tocopherol recovery. At a solvent-oil ratio of 4:1, the concentrate was not a great deal higher in tocopherol content than at a

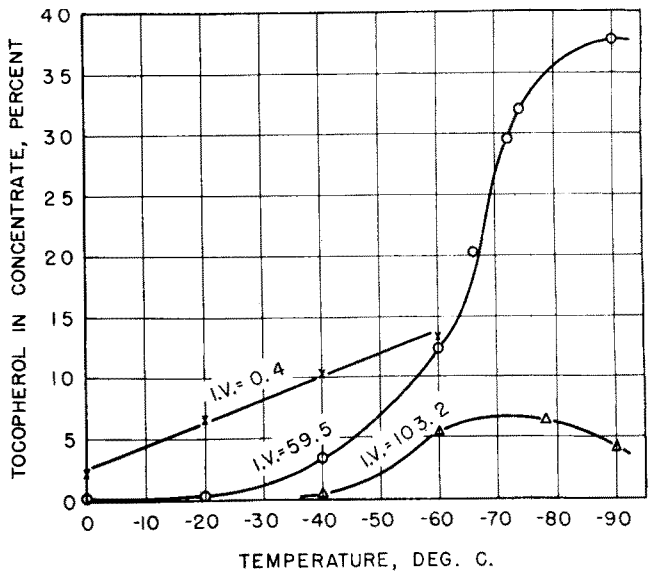

FIG. 2. Percent tocopherol present in concentrates of unhydrogenated, partially hydrogenated, and completely hydrogenated cottonseed oil at various temperatures of crystallization.

TABLE 2

Crystallization of Partially Hydrogenated Cottonseed Oil From Acetone at —60° C. to Produce Tocopherol Concentrates.

Ratio of solvent to oil, by weight	Yield of concentrate, gms. per 100 gms. oil	Tocopherol content of concentrate, percent	Recovery of tocopherols	
			Gms. per 100 gms. oil used	Percent recovery*
16:1	0.64	9.4	0.051	102
8:1	0.40	12.4	0.050	100
4:1	0.22	15.7	0.035	70

* The percent recovery in some cases exceeds 100, probably due to the limitations of the assay method.

ratio of 8:1, and the recovery of tocopherols was poor. A solvent-oil ratio of 8:1 may, therefore, be considered somewhere near the optimum.

Crystallization from Solvents Other Than Acetone

No effort was made to compare the many solvents available, with respect to their effectiveness in producing tocopherol concentrates. However, because of their ready availability and their usefulness in other crystallization processes, methyl ethyl ketone and Skellysolve B were tested in comparison with acetone. The results of these tests are detailed in Table 3. It

TABLE 3

Comparison Between Acetone, Methyl Ethyl Ketone, and Skellysolve B as Solvents for the Production of Tocopherol Concentrates From Hydrogenated Cottonseed Oil.

(All crystallizations carried out at —60° C. with 10 ml. of solvent per gm. of oil.)*

Solvent used	Oil used	Yield of concentrate, gms. per 100 gms. oil	Tocopherol content of concentrate, percent	Recovery of tocopherols	
				Gms. per 100 gms. oil used	Percent recovery†
Acetone	Partially hydrog.	0.40	12.4	0.050	100
Methyl ethyl ketone	Partially hydrog.	1.15	5.2	0.060	120
Skelly-solve B	Partially hydrog.	15.63	0.35	0.055	110
Acetone	Completely hydrog.	0.305	13.1	0.040	80
Skelly-solve B	Completely hydrog.	0.62	8.2	0.051	102

* In the case of acetone and methyl ethyl ketone, this corresponds to a solvent-oil ratio of 8:1, by weight. The ratio in the case of Skellysolve B is 6.3:1.

† The percent recovery in some cases exceeds 100, due probably to the limitations of the assay method.

will be seen that neither of these solvents produces concentrates as high in tocopherols as those obtained from acetone.

Direct Addition of Dry Ice to the Fat Solution

During the course of this work, a number of batches of fat and solvent were cooled by the direct addition of pulverized Dry Ice to the liquid, rather than by employing Dry Ice to chill an external bath. In every case where cooling was carried out in this manner, an abnormally low yield of tocopherols was obtained with the production of a concentrate correspondingly low in tocopherol content. The cause of this somewhat surprising effect is at present unknown, but is being further investigated.

Antioxygenic Activity of the Concentrates

The antioxygenic activity of fractionally crystallized concentrates from the different oils was tested by adding quantities equivalent to 0.03 percent tocopherols to lard and determining the stabilities of the mixtures. Results of the stability tests are shown in Table 4. For comparison, there are shown in the same table, the results of tests made on portions of the same lard to which had been added equivalent amounts of a molecularly distilled concentrate from the partially hydrogenated oil, and of pure α- and γ-tocopherols. The fractionally crystallized concentrates are all equal or superior in antioxygenic activity to the molecularly distilled concentrate from the partially hydrogenated oil. Hydrogenation of the oil appears to increase effectiveness of the concentrates.

TABLE 4

Antioxygenic Activity of Fractionally Crystallized Tocopherol Concentrates From Cottonseed Oil, in Comparison With a Molecularly Distilled Concentrate and Pure Tocopherols.

(Results expressed on the basis of stability of lard containing concentrate equivalent to 0.0312 percent tocopherols.)

Source of concentrate	Method of concentration	Stability*
Control	.....	2.0
Unhydrogenated oil	Fractional crystallization	7.8
Partially hydrogenated oil	Molecular distillation	7.4
Partially hydrogenated oil	Fractional crystallization	8.2
Completely hydrogenated oil	Fractional crystallization	10.0
Pure α-tocopherol	.....	8.5
Pure γ-tocopherol	.....	14.0

* Hours required to develop organoleptic rancidity at 110° C.

Summary

1. Tocopherol concentrates equivalent in tocopherol content and antioxygenic activity to molecularly distilled concentrates, have been obtained from cottonseed oil by hydrogenating the oil and removing the bulk of the glycerides and sterols by low temperature crystallization from acetone.

2. High-tocopherol concentrates can be obtained only from hydrogenated oils. Completely hydrogenated oils are the best source of concentrates at crystallization temperatures down to —60° C.; below this temperature partially hydrogenated oils are equally as good.

3. A solvent-oil ratio of 8:1 by weight appears to be about the optimum. At this ratio, crystallization from acetone at the temperature of Dry Ice (—78° C.) yields a concentrate containing 34 percent tocopherols from an oil originally containing 0.05 percent tocopherols.

4. The direct addition of Dry Ice to the solvent and oil is to be avoided, since this lowers the recovery of tocopherols.

5. Petroleum naphtha and methyl ethyl ketone are less suitable solvents than acetone, because of their greater capacity for dissolving glycerides at low temperatures.

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