

# Optical Activity of Butterfat and Vegetable Oils

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## Abstract

Optical activities of oils freshly expressed from viable seeds of rape, soybean and peanut show no change in rotation with time; neither do oils extracted from these materials by diethyl ether, as previously reported by other workers. Similarly, no evidence for racemization of butterfat isolated immediately after milking could be found. On removal of the steroids present, by chromatography on Celite impregnated with digitonin, and removal of the mono- and diglycerides by chromatography on alumina, butterfat maintained the highest degree of optical activity compared to most of the vegetable oils studied. This residual activity of butterfat tends to substantiate preferential location of short chain fatty acids on the 1 or 3 position of the triglycerides.

## Introduction

Reported studies of the optical activity of asymmetric triglycerides contain conflicting results. Suzuki et al. (1,2) claimed to have observed the racemization of oils extracted from viable seeds and freshly killed fish. Strangely no optical activity and subsequent racemization was observed in material isolated from dead seeds. Little or no optical activity should be expected from asymmetrically substituted triglycerides according to Schlenk (3,4) who also claimed no racemization of these materials occurred when held at room temperature.

Our own interest in the labile properties of butteroil led us to study its optical activity and the factors responsible for it. The same techniques were used to examine the rotatory power of some of the plant oils studied by Suzuki et al. The results we obtained are given in this paper.

## Materials and Methods

### Butteroil

Milk, immediately after removal from a cow, was mechanically separated and the cream so obtained was then quickly churned at a temperature of 10 C. The resulting butter was washed four times with cold distilled water, collected and converted into butteroil by warming to 39 C. Residual butter serum was removed from the oil by centrifuging for 5 min in a Servall bench-top centrifuge using a rotor speed of 7,660 rpm. Approximately 4 hr elapsed between milking and the time butteroil samples were ready for initial studies. Between studies butteroil samples were stored under nitrogen at a temperature of 5 C.

### Seed Oils

Oil samples were obtained from stocks of peanut, soy, sunflower and rape seeds whose viability had been established by preliminary germination studies. Both solvent extraction and pressing were used to obtain the oils.

Solvent extraction was carried out as described by Suzuki et al. (1) which, in brief, involved grinding the seeds to coarse powder in a Waring Blender, dissolving out their lipids with ethyl ether, filtering

off the insolubles, drying the filtrate with Na<sub>2</sub>SO<sub>4</sub> and removing the solvent by boiling under vacuum. Since the oils resulting from application of this procedure were slightly turbid, we cleared them by high speed centrifugation, as above, before any analyses were attempted.

Oils were pressed from seeds using a custom built, stainless steel piston and perforated cage having internal dimensions of 2¼ × 2¼ × 3¼ in. The piston was driven by a Carver Press and pressures ranging from 5,000 to 15,000 lb/in<sup>2</sup> were used to express oil from the seeds. A single pressing produced 10 to 25 g of oil depending on seed type. The crude oils were cleared of moisture by holding over Na<sub>2</sub>SO<sub>4</sub> then centrifuged clear using a Servall centrifuge as described above.

All oils were held at 5 C under nitrogen during storage.

### Sterol Removal

Sterols were removed from all oils using a 2.2 × 30 cm column packed with Celite 545 impregnated with an aqueous solution of digitonin as described by Schwartz et al. (5). Ten grams of packing material were used and so tamped that a flow rate of 0.5 to 0.75 ml hexane/min was obtained. A 7 g sample of oil dissolved in 21 ml of hexane was passed through the column to remove 3-β-hydroxy sterols. After washing the column with three 14 ml portions of hexane followed by three 14 ml portions of benzene, the eluate and washings were combined and freed of solvent by bubbling nitrogen through the mixture. The steroid free oils were dried over CaCl<sub>2</sub>.

### Mono- and Diglyceride Removal

Mono- and diglycerides were removed from sterol free oils by dissolving them in hexane and passing them through a column packed with a specially prepared alumina. The mono- and diglycerides adhere to the column and the residual oil is washed through and collected. After solvent removal by bubbling nitrogen through the mixture, the oils are ready for analyses. The method is to be reported in detail

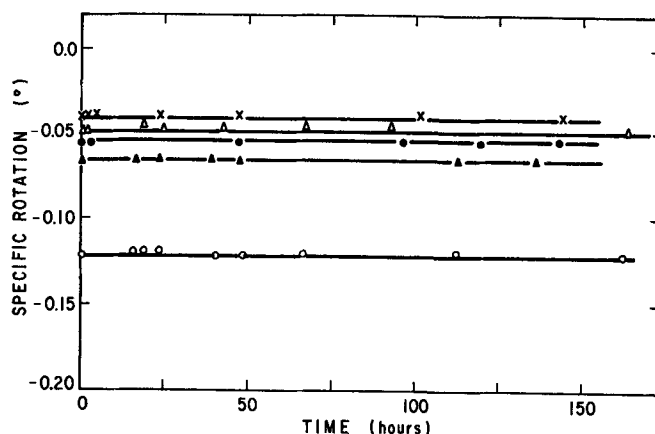


FIG. 1. Change in specific rotation of pressed seed oils and butteroil on holding. Temperature held at 37 C for first 24 hr and approximately 20 C thereafter. All rotations measured at 37 C. ×, butteroil; ●, peanut oil; Δ, soybean oil; ○, rapeseed oil; ▲, sunflower seed oil.

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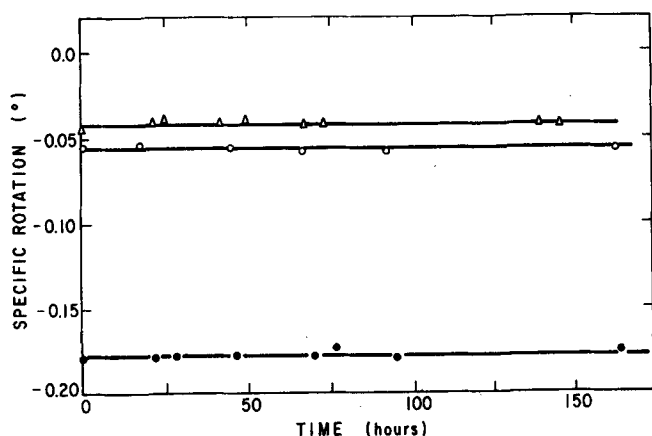


FIG. 2. Change in specific rotation of solvent extracted seed oils on holding. Temperature held at 37 C for first 24 hr and 20 C thereafter. All rotations measured at 37 C.  $\Delta$ , soybean oil;  $\circ$ , peanut oil;  $\bullet$ , rapeseed oil.

elsewhere according to its developer (Keeney, Chemistry Department, University of Maryland.)

#### Steam Deodorization

Hydroxy acids, lactones and related oxygenated material was removed from 275 g samples of butteroil by steam deodorization which was carried on for 6 hr at 170 C using the apparatus and technique described by Riemenschneider et al. (6). The deodorized oil was dried over  $\text{CaCl}_2$  before removal of sterols, mono- and diglycerides.

#### Measurement of Optical Rotation

The optical activity of pure oil samples was measured using a Perkin-Elmer Model 141 polarimeter equipped with digital readout, and a 1 dm water-jacketed polarimeter tube. Measurements were made at 37 C using light having a wave length of 589  $\mu\text{m}$ .

### Results

During the time period extending from 4 to 144 hr after milking, no evidence indicative of any racemization occurring in butteroil was obtained. The initial measured rotation of this material was  $-0.038^\circ$ . One hundred and forty four hours later the rotation measured  $-0.041^\circ$ .

Similar results were obtained in studies of the changes in optical rotation of oils pressed from seeds. The entire rotation data for these materials is shown in Figure 1. From this graph we can see no change with time in the optical activity of oils pressed from viable seeds.

Change in lipid isolation methodology did not affect this static pattern. From Figure 2 it can be seen that the lipids extracted from viable seeds by ethyl ether have low optical activities that remain constant with time.

TABLE I  
Effect of Sterol Removal and Mono- and Diglyceride Removal on Optical Activity of Natural Oils

Oil	[ $\alpha_D$ ] ( $^\circ$ )		
	Original	Without sterols	Without sterols and mono- and diglycerides
Butteroil	-0.040	+0.059	+0.043
Deodorized butteroil	-0.041	+0.043	+0.047
Soybean	-0.047	+0.020	+0.029
Peanut	-0.055	+0.015	+0.001
Rapeseed	-0.119	-0.10	-0.021
Sunflowerseed	-0.064	-0.022	+0.052

TABLE II  
Contributions of Oil Constituents to the Specific Rotation of the Natural Products

Oil	[ $\alpha_D$ ] ( $^\circ$ )		
	Triglycerides	Sterols	Mono- and diglycerides
Butteroil	+0.043	-0.099	+0.016
Soybean	+0.029	-0.067	-0.009
Peanut	+0.001	-0.070	+0.014
Rapeseed	-0.021	-0.109	+0.011
Sunflowerseed	+0.052	-0.086	-0.030

The observed optical activity of oils apparently results primarily from nontriglyceride constituents. In Table I are tabulated data showing the change in optical rotation of oils with removal of sterols, mono- and diglycerides. The contribution of each of these constituents to the observed optical rotation of the various oils is shown in Table II.

From the tables it can be seen that the sterols present in butteroil and plant seed oils can account for the bulk of their observed optical activity. Only the triglycerides of butteroil and sunflower seed oil have significant optical activity after the removal of sterols, mono- and diglycerides.

### Discussion

Our study of the optical rotation of freshly prepared butteroil and plant seed oils provided no data indicating racemization of triglycerides present as described by Suzuki et al. (2); neither was the rapid fluctuation of the optical activity of freshly extracted seed oil described by that author observed. Rotation, though weak, was observed in all oils studied but the values were all remarkably stable with increase in holding time.

We are unable to account for the variations between our observations and those of Suzuki et al. (1,2). He reports that oils extracted from dead seeds display no optical activity and no change with time. This might have accounted for the static situations we observed except that standard germination tests established the full viability of the seeds we used.

The data we obtained agrees with the conclusion drawn by Schlenk (4) that asymmetrically substituted triglycerides have very low optical activity unless radical differences exist in the chain length of the substitutes. In the case of peanut oil, all optical activity was lost when sterols, mono- and diglycerides were removed from the oil.

Significant positive rotatory power remains in butteroil and sunflower seed oil after removal of non-triglyceride optically active material. Since butteroil contains a relatively high concentration of short chain fatty acids, the residual optical activity of the triglyceride fraction of butteroil may be ascribed to their asymmetric distribution in the 1 or 3 positions. The location of the short chain fatty acids in these positions in the triglycerides of butteroil has been established in elegant fashion by Breckenridge and Kuksis (7). Our work serves as a rough physical confirmation of their findings.

### REFERENCES

- Suzuki, B., and Y. Inoue, Imperial Academy of Japan Proceedings 6, 71 (1930).
- Suzuki, B., Y. Inoue and R. Hata, Ibid. 1, 222 (1931).
- Schlenk, W., Festschr. Carl Wurster 60 Geburtstag, 105 (1960).
- Schlenk, W., Jr., JAOCS 42, 945 (1965).
- Schwartz, D. P., G. R. Brewington and L. H. Burgwald, J. Lipid Res. 8, 54 (1967).
- Riemenschneider, R. W., S. F. Herb, E. M. Hammaker and F. E. Luddy, Oil Soap 21, 307 (1944).
- Breckenridge, W. C., and A. Kuksis, J. Lipid Res. 9, 388 (1968).

[Received February 24, 1969]