# Nutritional Studies of Partially Hydrogenated Rapeseed Oil

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In three experiments rats were fed a purified basal diet with 20% by weight of partially hydrogenated rapeseed oil. One sample promoted greater weight gains than the unhydrogenated oil. Another sample, containing a higher concentration of octadecadienoic acids other than 9,12-linoleic acid, produced the same response as the unhydrogenated material. With other samples of hydrogenated rapeseed oil, possessing less linoleic acid but other octadecadienoic acids, significantly lower weight gains were obtained. The alterations in the $C_{18}$ fatty acids resulting from hydrogenation of rapeseed oil appeared to be responsible for differing responses in weight gain.

THERE IS A SCARCITY of information on the nutritional aspects of partially hydrogenated rapeseed oil. In a series of studies in Sweden (1) it was found that rats maintained for about three years on butter or margarine did not differ in growth, longevity, or reproductive performance while the margarine during the experimental period contained 0 to 47.5% hydrogenated rapeseed oil. Carroll (2) reported that hydrogenation destroyed the growth-depressing action of the oil.

Since some rapeseed oil which may be consumed as food is partially hydrogenated, studies were undertaken on several industrially-processed samples.

## Methods

In each experiment, weanling Wistar rats of the Food and Drug colony were grouped according to initial body weight (30–48 g.) in a randomized block design and housed in individual, screen-bottomed cages. The diet was supplied *ad libitum* and consisted of the following in percentage by weight: test oil, 20; casein, 19; vitamin mixture in casein, 1; corn starch, 30; sucrose, 20; salt mixture, U.S.P. XIV, 4; alphacel, 6. Each 100-g. vitamin mixture contained: 100 mg. of thiamine, 100 mg. of riboflavin, 100 mg. of pyridoxine HCl, 300 mg. of calcium pantothenate, 5 g. of inositol, 500 mg. of niacin, 1 g. of p-aminobenzoic acid, 2 mg. of biotin, 0.2 mg. of vitamin $B_{12}$, 10 g. of choline chloride, 50,000 I.U. of vitamin A, 10,000 I.U. of vitamin D, 1,000 I.U. of vitamin E.

In the first experiment 120 male rats received unhydrogenated or partially hydrogenated soy oil or rapeseed oil alone or in the combinations shown in Table I. The melting point of the hydrogenated soy oil was 38°C. and that of the rapeseed oil, 42°C. During the third week of the test all feces were collected and frozen until their content of free fatty acids and neutral fat was determined as previously described (3). After eight weeks the animals were anaesthetized with ether and bled; the liver and adrenals were removed and weighed.

The second experiment comprised 15 male and 15 female rats fed unhydrogenated rapeseed oil as used in the previous experiment, and the same number of each sex were fed partially hydrogenated rapeseed oil of m.p. 40°C. The animals were maintained on their respective regimens for six weeks.

In the third experiment rapeseed oil which had been partially hydrogenated by conditions used in shortening manufacture and randomly rearranged, (m.p. 39.0°C.), rapeseed oil which had been partially hydrogenated by conditions used in margarine manufacture (m.p. 41.5° C.), unhydrogenated rapeseed oil,[1] and corn oil were each fed to 10 male rats for six weeks. At the termination of the test, livers, testes, and adrenals were weighed, and the concentration of serum cholesterol was determined by the method of Henly (4) and of liver lipids by the method of Folch (5).

The fatty acid composition of oils was determined by gas-liquid phase chromatography, using units previously described (6). Succinate-1, 4-butanediol, m.p. 114–115°C., was prepared from equal quantities of reagent grade 1, 4-butanediol and succinic acid. Six grams of the polyester were dissolved in 110 ml. of chloroform, to which were added 36 g. of 60–80 mesh, acid-washed $C_{22}$ firebrick. The mixture was allowed to stand at room temperature for 15 min. with occasional stirring, then was transferred to a shallow, stainless steel tray in a well-ventilated fume hood, where it was stirred continuously with a spatula until visibly dry. The mixture was dried in a vacuum oven at 80°C. to remove the last traces of solvent. Lack of agglomeration was taken as an indication of satisfactory coating of the polyester on the firebrick. The packed copper column, 8 ft. long and 3/16 in. in diameter, was operated at 208°C., measured by a thermocouple on the column or a thermometer at the level of the column, and at a helium flow rate of 60 ml. per minute measured by a soap bubble meter at the column exit. The injector temperature was 235°C.; the bridge current was 250 ma on Gow Mac filaments; the recorder response was 2.5 mv. Sample sizes were 0.25 $\mu$l. for rapeseed esters and at 0.15 $\mu$l. for soy esters. A silicone column was used for the rapeseed oils to check the analyses obtained by the polyester column.

## Results

As shown in Table I, the weight gains obtained at four and eight weeks in the first experiment were greatest when the predominant dietary fat was soy oil in either the hydrogenated or unhydrogenated

[1] The rapeseed oil consisted of the following fatty acids:
$C_{16}$ 4.1% $C_{18:1}$ 18.8% $C_{18:3}$ 9.5% $C_{20:2}$ 0.4%
$C_{18:0}$ 1.9% $C_{18:2}$ 16.3% $C_{20:1}$ 11.6% $C_{22:1}$ 37.0%

TABLE I

Experiment I

Weight Gain and Food Consumption of Rats Fed 20% Unhydrogenated Soy Oil (US), Hydrogenated Soy Oil (HS), Unhydrogenated Rapeseed Oil I (UR), and Hydrogenated Rapeseed Oil (HR)
(10 rats per group)

| Portions of Oil | | | | Weight Gain | | Food Cons. | | Wt. gain, 8 wks., adjusted for food cons. |
|---|---|---|---|---|---|---|---|---|
| US | HS | HR | UR | 4 wks. | 8 wks. | 4 wks. | 8 wks. | |
| | | | | g. | g. | g. | g. | g. |
| 3 | .... | .... | .... | 110 | 227 | 253 | 596 | 202 |
| 2 | 1 | .... | .... | 117 | 222 | 252 | 590 | 200 |
| 2 | .... | 1 | .... | 103 | 210 | 238 | 573 | 194 |
| 1 | 2 | .... | .... | 116 | 201 | 245 | 559 | 192 |
| .... | 2 | .... | 1 | 108 | 195 | 212 | 548 | 192 |
| .... | 1 | .... | 2 | 103 | 185 | 228 | 529 | 189 |
| .... | 3 | .... | .... | 82 | 184 | 251 | 571 | 170 |
| 1 | .... | 2 | .... | 99 | 183 | 240 | 536 | 184 |
| .... | .... | 2 | 1 | 95 | 178 | 212 | 511 | 190 |
| .... | .... | 3 | .... | 82 | 174 | 191 | 511 | 186 |
| .... | .... | 1 | 2 | 70 | 163 | 200 | 477 | 190 |
| .... | .... | .... | 3 | 74 | 151 | 213 | 463 | 184 |

state. From the first week of the experiment to its termination it was found that the hydrogenation of rapeseed oil increased its growth-promoting action whereas hydrogenation of the soy decreased it. At eight weeks the difference in weight necessary for significance at $P = 0.05$ was approximately 20 g. An adjustment of weight gain for food consumption by a co-variance analysis indicated that the hydrogenated soy oil was the most poorly utilized. It was interesting to note the similarity in adjusted weight gains obtained with the 1:2 mixture of unhydrogenated soy oil and hydrogenated soy oil and with the 1:2 mixture of unhydrogenated rapeseed oil and hydrogenated rapeseed oil.

The weekly excretion of free fatty acids and neutral lipid material per rat fed unhydrogenated soy oil, hydrogenated soy oil, hydrogenated rapeseed oil, and unhydrogenated rapeseed oil was 0.114 g., 0.179 g., 0.279 g., and 0.438 g., respectively. Rats ingesting either of the soy oils excreted significantly less fat than those consuming unhydrogenated rapeseed oil.

There were no significant differences in the absolute weight of the livers or the adrenals although the body weights differed appreciably. With the second sample of hydrogenated rapeseed oil no differences in weight gain or food consumption were observed in either sex (Table II).

TABLE II

Experiment II

*Body Weight and Food Consumption of Rats of Both Sexes Fed 20% Hydrogenated Rapeseed Oil II (HR) and Unhydrogenated Rapeseed Oil (UR) (15 male and 15 female rats per group)*

| Dietary Oil | Body Weight | | Food Cons. | |
|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ |
| | *g.* | *g.* | *g.* | *g.* |
| HR | 155 | 106 | 369 | 281 |
| UR | 159 | 110 | 344 | 297 |

As shown in Table III, the differently processed samples of hydrogenated rapeseed oil had similar effects on food consumption, body and organ weights, and the concentration of liver lipids, but the level of serum cholesterol was lower ($P = 0.05$) in the animals fed the randomly rearranged hydrogenated oil. Body, testes, and adrenal weights, the concentration of serum cholesterol and of liver lipids were significantly lower in the animals receiving the hydrogenated oil than in those receiving either of the unhydrogenated oils. The finding that the unhydrogenated oils produced higher serum cholesterol levels than the hydrogenated oils was in agreement with the work of Swell and Flick (7), who showed that rats fed lard had a higher level of blood cholesterol than those fed stearic acid. As found previously (3), a higher food consumption, body weight, and testes weight were obtained in the rats receiving corn oil than in those receiving unhydrogenated rapeseed oil.

A gas chromatogram, Figure 1, illustrates the separation of the methyl esters of fatty acids of partially hydrogenated rapeseed oil with its three octadecadienoic acids. Proof that the second octadecadienoic acid was the natural isomer was obtained by comparison of the emergence time of linoleic acid in nonhydrogenated oils. In Table IV is shown the fatty acid composition of the partially hydrogenated samples of rapeseed oil. Hydrogenated rapeseed oil which increased weight gain (Experiment I) was relatively high in linoleic acid and low in other octadecadienoic acids, and that which did not alter the body weight

TABLE III

Experiment III

Data for Rats Fed 20% Hydrogenated Rapeseed Oils (HR-A and HR-B), Unhydrogenated Rapeseed Oil (UR), or Unhydrogenated Corn Oil (UC) *(10 rats per group)*

| Dietary oil | Food consumption | Body weight | Liver weight | Testes weight | Adrenal weight | Serum cholesterol | Liver lipids |
|---|---|---|---|---|---|---|---|
| | *g.* | *g.* | *g.* | *g./pair* | *mg./pair* | *mg./100 ml.* | *%* |
| HR-A[a] | 267 | 113 | 5.19 | 1.45 | 18.1 | 73.4 | 5.08 |
| HR-B[b] | 276 | 114 | 6.10 | 1.49 | 20.3 | 94.0 | 4.72 |
| UR | 300 | 144 | 6.53 | 1.97 | 26.4 | 126.8 | 6.19 |
| UC | 415 | 199 | 7.82 | 2.48 | 27.4 | 134.0 | 6.63 |

[a] Hydrogenated by conditions used in shortening manufacture and randomly rearranged.

[b] Hydrogenated by conditions used in margarine manufacture.

from that of animals fed the unhydrogenated rapeseed oil (Experiment II) was high in octadecadienoic acids I and III. In the other samples of hydrogenated rapeseed oil (Experiment III) octadecadienoic acid II (linoleic acid) was below 2% of the dietary fatty acids and was consumed at a level of less than 30 mg. per day instead of the preferred level of 100 mg. per day (8), and the other octadecadienoic acids were prominent. Under these conditions the weight gains were significantly lower after hydrogenation of the rapeseed oil.

## Discussion

A slower rate of growth of rats fed hydrogenated soy oil instead of the corresponding unhydrogenated oil was expected in view of the findings of Aaes-Jorgensen and Dam (9) that 7% hydrogenated peanut oil (m.p. 35–37°C.) had a growth-depressing action compared with the unhydrogenated oil. Since food consumption was normal, the decreased growth rates appeared to be due to poorer utilization. Sahasrabudhe and Subrahmanyan (10) showed that the melting point of hardened peanut oil and the coefficient of absorption were inversely related. It was of particular interest that in the first experiment hydrogenation of oil containing erucic acid improved its growth-promoting quality. Carroll (2) had also found that hydrogenation of this oil partially eliminated its growth-depressing characteristics. That this effect of hydrogenation of rapeseed is not consistent was shown in the experiments.

Since the growth rate of rats fed hydrogenated oils could be improved by the addition of linoleic acid (8,11,12), *it is apparent that its disappearance during* hydrogenation contributed to the lower body weight of the animals studied here. There is also evidence that saturated fat increases the requirement for linoleic acid (13), particularly when the triglycerides contain long-chain fatty acids (14). The

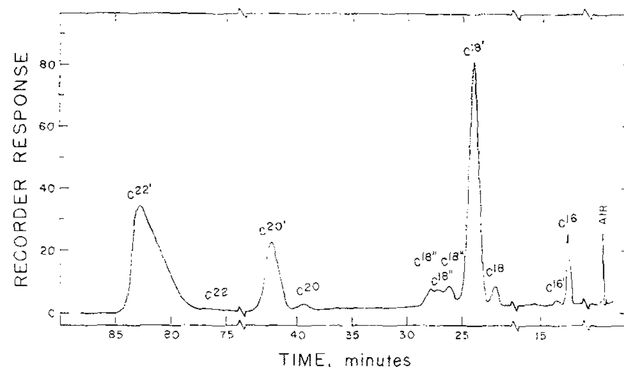


FIG. 1. Gas chromatogram of partially hydrogenated rapeseed oil.

TABLE IV

Fatty Acid Composition of the Partially Hydrogenated Samples of Rapeseed Oil Studied in Three Experiments (% by weight)

| Fatty acid | Exp. I | | Exp. II | | Exp. III | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | A[c] | | B[d] | |
| | P.E.[a] | Si[b] | P.E. | Si | P.E. | Si | P.E. | Si |
| $C_{12:0}$ | trace | trace | trace | trace | trace | trace | trace | trace |
| $C_{14:0}$ | trace | trace | trace | trace | trace | trace | trace | trace |
| $C_{16:0}$ | 4.0 | | 4.1 | | 3.7 | | 3.5 | |
| $C_{16:1}$ | 0.3 | | 0.4 | | 0.6 | | 0.5 | |
| Total $C_{16}$ | 4.3 | 4.1 | 4.5 | 4.0 | 4.3 | 3.9 | 4.0 | 3.6 |
| $C_{18:0}$ | 8.2 | | 6.4 | | 6.2 | | 7.2 | |
| $C_{18:1}$ | 34.2 | | 34.8 | | 37.6 | | 37.2 | |
| $C_{18:2}$ I | 1.6 | | 4.6 | | 3.7 | | 3.4 | |
| $C_{18:2}$ II[e] | 4.7 | | 3.1 | | 1.8 | | 1.9 | |
| $C_{18:2}$ III | 1.4 | | 2.5 | | 1.2 | | 1.0 | |
| $C_{18:3}$ I[e] | .... | | .... | | 0.3 | | 0.2 | |
| $C_{18:3}$ II | 0.8 | | .... | | .... | | .... | |
| Total $C_{18}$ | 50.9 | 50.4 | 51.4 | 52.7 | 50.8 | 50.7 | 50.9 | 51.2 |
| $C_{20:0}$ | 2.5 | | 1.7 | | 2.2 | | 2.1 | |
| $C_{20:1}$ | 11.3 | | 10.9 | | 10.3 | | 10.5 | |
| Total $C_{20}$ | 13.8 | 14.5 | 12.6 | 12.7 | 12.5 | 12.5 | 12.6 | 12.8 |
| $C_{22:0}$ | 3.8 | | 3.6 | | 2.8 | | 4.1 | |
| $C_{22:1}$ | 27.2 | | 27.9 | | 29.6 | | 28.4 | |
| Total $C_{22}$ | 31.0 | 31.0 | 31.5 | 30.6 | 32.4 | 32.9 | 32.5 | 32.4 |

[a] Butanediol-succinate polyester column.
[b] Silicone column.
[c] Hydrogenated by conditions used in shortening manufacture and randomly rearranged.
[d] Hydrogenated by conditions used in margarine manufacture.
[e] Natural isomer.

other factor of apparent significance in affecting body-weight gain of rats fed hydrogenated rapeseed oil was the occurrence of isomers of linoleic acid, possibly 9,15-octadecadienoic and 12,15-octadecadienoic acids derived from linolenic acid. Geometric isomers of linoleic acid were found to have no effect on essential fatty acid activity (15,16), but positional isomers were not similarly investigated. From these studies it is shown that hydrogenation of rapeseed oil increases, does not change, or decreases the growth-promoting qualities of rapeseed oil, depending at least in part on the final fatty acid composition. Low gains in weight were associated with a decrease of natural linoleic acid and the presence of other octadecadienoic acids.


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# Determination of Mono-, Di-, and Triglycerides by Molecular Distillation and Thin-Layer Chromatography[1,2]


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Analysis of mixtures of mono-, di-, and triglycerides by molecular distillation and thin-layer chromatography is described. Mono- and diglycerides undergo appreciable acyl migration through the effect of heat during molecular distillation. Nevertheless this technique may be used for the quantitative analysis of mixtures of mono-, di-, and triglycerides, provided there are no substances present which catalyze disproportionation.

Thin-layer chromatographic (TLC) analysis of mono-, di-, and triglycerides is fast and simple and can be carried out on a micro-scale with a high degree of accuracy and precision. It also is extremely sensitive, permitting the quantitative estimation of as little as 0.1% of a single component in a mixture.

The routine analyses of α- and β-monoglycerides and of 1,2- and 1,3-diglycerides also may be performed by this method.



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[2] Presented at fall meeting, American Oil Chemists' Society, New York, October 17–19, 1960.


THE ANALYSIS of mixtures of mono-, di-, and triglycerides is usually carried out by column chromatographic procedures, employing selective adsorption (1,6) or partition techniques (12). In general, these methods are time-consuming and involved, since many fractions must be collected and analyzed for a single determination. Furthermore the precision and accuracy of analyses by these methods, in general, is less than desirable for many analytical purposes.

Described are two procedures for the analysis of