Nutritional Evaluation of Some Heated Oils[1,2]

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THE TOXIC NATURE of heat-abused oils has been the subject of much discussion in the literature. Fractionation of these oils has yielded various polymerized products which, when fed to rats, have resulted in interferences with good nutrition and the appearance of pathological symptoms.

Polymerized oils have been prepared in many ways, by different methods of treating the oils and by using various types of oils as starting materials. Many conflicting reports of the impaired nutrition resulting from the ingestion of these polymers are available. Crampton and co-workers (1) heated soybean and sunflower seed oil anaerobically for 24–26 hrs. and reported the production of a toxic substance resulting from the polyunsaturated nature of the fatty acids of these oils. Johnson and co-workers (2), in experiments in which rats were fed diets containing heated corn oil, heated margarine, and heated butter fat, confirmed the observation that the toxic effect of the heated oils resulted from their polyunsaturated fatty acid content since in animals fed the heated corn oil the growth rate was considerably lower than in the control animals receiving fresh oil; in animals fed heated margarine only moderately reduced growth was observed as compared with the control animals whereas the ingestion of heated butter fat had no deleterious effect on growth. In all of these groups however no permanent metabolic damage was produced. Experiments in which heat-polymerized menhaden oil and linseed oil were fed to rats also resulted in the observation that the toxicity was associated with polymerized polyunsaturated fatty acids contained in these oils (3).

These interferences with nutrition can be remedied. In experiments reported by Kaunitz (4) the toxicity induced in rats by feeding a heat-treated oxidized cottonseed oil was partially nullified by the addition of fresh oil to the diet. Other investigators reported that the toxicity of thermally polymerized fats could be partially overcome by supplementation with pyridoxine (5).

Recently studies have become available which indicate that the laboratory heat-treatment of oils, on which the above reports are based, are unrealistically drastic. Melnick (6) has reviewed critically these earlier reports and has concluded that the findings are irrelevant to practical operations. Thus, in a survey by Melnick and associates (6–8) of operations by 86 different potato chip manufacturers, it was shown that the frying oils and fats used in these operations had only a 1% decrease in iodine value, which was of no nutritional significance. Results from our laboratory have shown that heated margarine fat caused no decrease in nutritional value in tests conducted on rats (9). Rice and co-workers (10) reported studies of 34 samples of used fats obtained from restaurants, bakeries, and potato-chip producers and showed that even the most severe usage caused only slight decreases in caloric availability and slight increases in liver size in tests with rats.

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[2] Contribution No. 467.

TABLE I
Description of Oils

Oil or fat	Conditions of heating	I.V.	Decrease in I.V. resulting from heating %	Viscosity (centistokes at 27°C.)
Soybean oil	....	128	....	52
Soybean oil$_{L.P.}$	610°F. 70 min.	120	6	92
Soybean oil$_{H.P.}$	610°F. 100 min.	116	10	114
Cottonseed oil	....	112	....	55
Cottonseed oil$_H$	610°F. 70 min.	106	5	83
Lard	400°F. 60 min.	61	....	67
Lard$_H$	610°F. 70 min.	59	3	78

The investigation reported here was undertaken to evaluate the nutritional quality (and safety) of several heated fats, containing different concentrations of polyunsaturated fatty acids, using unheated samples as controls. The investigation was limited to a study of thermal nonoxidative polymers prepared under rigid laboratory conditions. It was desired to relate changes in physical constants, such as viscosity and iodine value, with several nutritional indices. It has been our experience that diets which seem to be adequate for growth and maintenance may be quite unsatisfactory for reproduction where different types of stresses are brought to bear on the animal. The survival of groups of these animals was followed over their life span, and when a 75% mortality was reached in each group, the animals were sacrificed and analyses of tissues for cholesterol were conducted.

Experimental

Soybean oil, cottonseed oil, and lard[3] were heated as described below. The changes in various physical constants are shown in Table I. Heat was applied to these samples *in vacuo* to reduce peroxide formation.

[3] The soybean and cottonseed oils were commercially refined and were purchased through regular trade channels. The lard was purchased from Armour and Company and was refined and bleached in the pilot plant of the Glenview Laboratories of the National Dairy Products Corporation. All heated oils were subject to deaeration since this is part of the deodorization process.

TABLE II
Basal Diet

Constituent	% of Diet
Casein	24
Cellulose	4
Salt mixture[a]	4
Fat	15
Sucrose	52.5
Choline	0.24
Dry vitamin mix[b]	0.19
Mixed tocopherols (34%)	0.03
Vitamin A	10,000 units/kg diet
Vitamin D	2,000 units/kg diet
Vitamin B_{12}	60 µg/kg diet

[a] Wesson modification of Osborne-Mendel salt mixture.
[b] Dry Vitamin Mix: Thiamin HCl 4.59, Riboflavin 1.72, Niacin 3.82, Folic acid 0.638, Pyridoxine 1.72, Ca pantothenate 3.82, Menadione 0.319, Biotin 0.159, Vitamin C 12.7, P-aminobenzoic acid 38.6, Inositol 31.9.

TABLE III

Food Consumption, Food Efficiency, and Digestibility of Various Heated and Unheated Fats

Diet	S.O.	S.O.L.P.	S.O.H.P.	Lard	LardH	CSO	CSOH
Food consumption (g.)							
Weeks 1– 2	63	69	63	72	65	66	67
3– 4	93	95	88	91	97	95	97
5– 6	105	103	107	113	100	109	107
7– 8	103	104	103	119	114	115	107
9–10	97	105	103	108	103	96	96
Food efficiency							
Gain in wt./g. food eaten							
Week 2	0.63	0.47	0.49	0.43	0.53	0.50	0.58
4	0.36	0.31	0.30	0.33	0.33	0.36	0.37
6	0.27	0.24	0.28	0.16	0.17	0.23	0.23
Digestibility (%)	98	93	91	93	94	96	93

Values are the averages of results obtained from 15 male rats per group.

The control soybean oil (S.O.) was deaerated and packed directly into gallon cans which had been flushed with nitrogen. The low-polymer soybean oil ($S.O._{L.P.}$) was heated at 610°F. for 70 min. at a pressure of 2 millimeters. A small amount of steam was introduced for agitation. The oil was then cooled and packed directly into nitrogen-flushed cans. The high-polymer soybean oil ($S.O._{H.P.}$) was treated similarly except for the longer heating period, 100 min.

The control cottonseed oil (CSO) was treated similarly to the control soybean oil. The treatment and handling of the heated cottonseed oil (CSO_H) was identical to that given the low-polymer soybean oil.

The control lard was 100% leaf lard which, after refining and bleaching, was subjected to a light deodorization at 400°F. for 60 min. at 2 mm. of pressure. The heated lard product was obtained from the control lard by processing under conditions identical with those used for the low-level polymer soybean oil.

All fats were included in the diet at the level of 15% (Table II). The diets were fed to groups of 30 male and 30 female rats from weaning.

TABLE IV

Reproductive Performance of Female Rats Fed Heated and Unheated Cottonseed Oil and Heated and Unheated Lard

Category	CSO	CSOH	Lard	LardH
Females bred	14	14	14	14
Litters cast (%)	93	100	72	72
Litters (at birth)				
Total No. rats	124	153	80	93
No./litter	9.5	10.9	8.0	9.3
Litters (at 3 days)				
Total No. rats	91	101	59	52
Av. wt./rat	6.6	7.0	6.7	6.4
Litters (at 21 days)[a]				
Total No. rats	56	63	53	38
Av. wt./rat	34.5	31.9	33.8	31.4
% Mortality				
0– 3 days	27	27	36	44
4–21 days	20	15	2	7

[a] Litters were cut to seven at three days.

Results and Discussion

Food consumption was measured during the first 10 weeks, and food efficiency was calculated for six weeks. The results are shown in Table III. No significant differences were observed in the animals fed the heated fats as compared with the control animals fed the unheated fats. Digestibility measurements however indicated that small decreases in digestibility were obtained with the heated soybean and cottonseed oils (Table III).

Growth in all groups in both male and female rats was little affected by the presence of the heated fats in the diet (Figure 1). The gain in weight is charted over a four-month period during the active growth of the animals. Although only a slight depression in growth in the animals fed the diet containing the highly polymerized soybean oil was observable at the end of four months, the interference with growth in this group became more pronounced with time.

TABLE V

Reproductive Performance of Female Rats Fed Unheated Soybean Oil and Heated Soybean Oil with and without Supplements of Linoleate or α-Tocopherol

Category	S.O.	S.O.L.P.	S.O.H.P.	S.O.H.P. + Linoleate	S.O.H.P. + α-tocopherol
Females bred	15	15	15	15	15
Litters cast (%)	100	93	80	87	100
Litters (at birth)					
Total No. rats	159	141	110	131	176
No./litter	10.6	10.1	9.2	10.1	11.7
Litters (at 3 days)					
Total No. rats	141	121	72	120	157
Av. wt./rat	7.3	6.9	6.8	6.7	7.3
Litters (at 21 days)[a]					
Total No. rats	96	88	59	69	95
Av. wt./rat	33.4	32.9	34.6	38.4	36.5
% Mortality 0– 3 days	11	16	34	8	11
4–21 days	2	15	0	18	10

[a] Litters were cut to seven at three days.

When male rats on the experimental diets were mated with female rats of our stock colony, there was no indication of any interference with gonadal development or maturation of sperm. Reproduction and lactation were normal. However when female rats on experimental diets were mated with male rats of our stock colony, although no significant differences in reproductive performance were obtained with animals fed unheated cottonseed oil as compared with the heated oil or unheated lard as compared with heated lard (Table IV), an interference with reproduction was observed in the animals fed the highly polymerized soybean oil (Table V). This was most evident in the number of young born and in the survival of the young at three days. In an attempt to determine the nature of this interference with pregnancy and lactation, female rats on the high-polymer soybean oil diet were supplemented from weaning with either 100 mg.

TABLE VI

Time in Weeks at Which 25, 50, and 75% Mortality of Male and Female Rats, Fed Heated and Unheated Fats, Was Reached

Diet		LD_{25} Wks.	LD_{50} Wks.	LD_{75} Wks.	Incidence of cysts and tumors
S.O.	M	75	97	116	0
	F	87	99	116	4
S.O.L.P.	M	75	108	115	0
	F	87	101	115	6
S.O.H.P.	M	82	100	117	1
	F	60	97	116	4
CSO	M	57	91	112	3
	F	77	107	112	9
CSOH	M	82	110	116	2
	F	87	97	115	3
Lard	M	65	97	116	0
	F	60	88	111	7
LardH	M	72	108	115	1
	F	82	109	114	6

TABLE VII
Cholesterol Levels in Plasma and Liver in Aged Male Rats Fed Various Fat Diets

Diet	Age at sacrifice[a]	Wt. at sacrifice	Plasma			Wt. % of body wt.	Liver		
			Cholesterol				Cholesterol		Lipid
			Free[b]	Total[b]	% F.		Free[b]	Total[b]	Total[b]
	weeks	*g.*	*mg. %*	*mg. %*			*mg./g.*	*mg./g.*	*mg./g.*
S.O.	116(5)	433	24 ± 1	81 ± 5	32	2.3	2.3 ± 0.1	3.6 ± 0.3	52.5 ± 6.1
S.O.L.P.	115(6)	470	28 ± 5	67 ± 8	42	2.4	2.2 ± 0.1	3.2 ± 0.4	56.9 ± 4.3
S.O.H.P.	117(6)	389	26 ± 2	78 ± 6	33	3.1	2.5 ± 0.1	3.4 ± 0.4	46.4 ± 6.1
Lard	116(6)	451	25 ± 3	80 ± 3	31	2.5	2.0 ± 0.1	3.0 ± 0.2	50.9 ± 2.8
LardH	111(6)	466	31 ± 3	88 ± 5	35	2.4	2.0 ± 0.1	2.9 ± 0.1	40.3 ± 1.7
CSO	116(3)	380	41 ± 9	108 ± 7	33	2.5	2.5 ± 0.1	3.0 ± 0.1	48.8 ± 10.0
CSOH	116(6)	415	33 ± 2	84 ± 10	40	2.6	2.2 ± 0.1	2.8 ± 0.2	41.2 ± 2.2

[a] Numbers in parentheses are numbers of animals in each group.
[b] Including standard error of the mean.

of the essential fatty acid ester, linoleate, or with twice the amount of α-tocopherol normally included in the diet. These animals were then bred with males of our stock colony as before. Although a slight improvement in reproductive performance was noted as a result of linoleate supplementation, the addition of increased Vitamin E yielded results comparable to those obtained with the unheated oil, indicating that the ingestion of this highly polymerized soybean oil increased the requirement for this vitamin.

The survivals of rats fed the heated oils and the fresh oils were practically the same in all cases (Table VI). A 75% mortality was obtained between 114 and 117 weeks. There was no evidence of increased tumor incidence in the groups ingesting the heated oils.

The results of the cholesterol determinations in plasma and liver of the surviving male and female animals are shown in Tables VII and VIII. Nishida *et al.* (11) have reported a depression in serum cholesterol levels as a result of the substitution of heated oil for fresh oil in the diet. Similar results were evident only in female rats fed the diet containing heated lard and in male rats ingesting the heated cottonseed oil diet. In all the other groups plasma cholesterol levels were unaffected by the substitution of heated for unheated fats.

The concentration of cholesterol in liver of these animals was very similar in all cases. Total lipid values were similarly unchanged by the substitution of heated fats for unheated fats in the diet. However the weight of the liver of male rats fed the highly polymerized soybean oil, expressed as percentage body weight, was higher than in the other groups; this increase was not reflected by elevations in cholesterol and total lipid but resulted from the lower body weight observed in these animals.

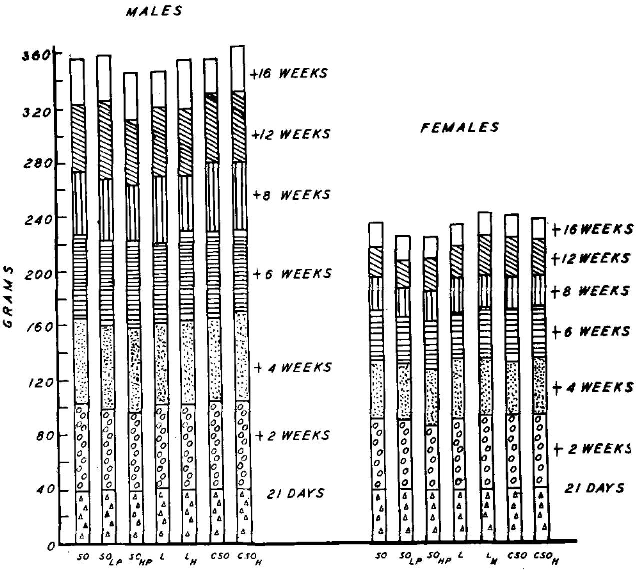

FIG. 1. Growth over a four-month period of male and female rats fed from weaning various unheated and heated fats.

Summary

Soybean oil, cottonseed oil, and lard were heated *in vacuo* for either 70 and/or 100 min. at 610°F. and were fed to male and female rats at a level of 15% in the diet over a complete life span. Nutritional indices such as growth, including food efficiency and digestibility of the fats, reproduction and lactation, longevity, and tissue cholesterol and total lipid were measured. No evidence of impaired nutrition or harmfulness was observed in any group except in those fed the more highly polymerized soybean oil, where a decrease in iodine value of 10% and a 100% increase in viscosity was associated with a slight depression in growth and an interference with reproductive performance in the female animal. This interference with the reproductive process was alleviated by supplementation with α-tocopherol. There was no evidence of increased tumor incidence in the groups ingesting the heated oils. Digestibility of the heated soybean and cottonseed oils was only slightly reduced.

TABLE VIII
Cholesterol Levels in Plasma and Liver in Aged Female Rats Fed Various Fat Diets

Diet	Age at sacrifice[a]	Wt. at sacrifice	Plasma			Wt. % of body wt.	Liver		
			Cholesterol				Cholesterol		Lipid
			Free[b]	Total[b]	% F.		Free[b]	Total[b]	Total[b]
	weeks	*g.*	*mg. %*	*mg. %*			*mg./g.*	*mg./g.*	*mg./g.*
S.O.	116(5)	288	22 ± 1	67 ± 4	33	3.0	2.2 ± 0.1	2.8 ± 0.2	45.0 ± 5.7
S.O.L.P.	115(6)	348	23 ± 2	86 ± 5	26	3.2	2.1 ± 0.1	2.6 ± 0.1	42.2 ± 3.3
S.O.H.P.	116(6)	343	34 ± 4	85 ± 11	41	2.8	2.4 ± 0.1	2.6 ± 0.1	40.1 ± 2.4
Lard	115(6)	362	31 ± 3	104 ± 5	30	2.7	2.1 ± 0.1	2.5 ± 0.2	42.8 ± 4.2
LardH	116(4)	370	21 ± 1	52 ± 2	40	2.8	2.2 ± 0.1	2.9 ± 0.2	46.2 ± 6.5
CSO	112(6)	388	30 ± 2	80 ± 6	38	3.0	1.9 ± 0.1	2.4 ± 0.1	34.5 ± 1.6
CSOH	115(4)	418	29 ± 5	77 ± 12	39	2.4	2.2 ± 0.2	3.1 ± 0.2	53.6 ± 2.8

[a] Numbers in parentheses are numbers of animals in each group.
[b] Including standard error of the mean.

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Fatty Acid Composition of Several Varieties of Soybeans[1]

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LITTLE INFORMATION has been published on the effects of different crop years and widely spaced geographic locations on the percentage of fatty acids in soybean oil. Fatty acid composition of soybean oil for 13 varieties was reported by Alderks (1) and for a single variety by Simmons and Quackenbush (8). Several others also have reported fatty acid data based on single or composite samples.

Such studies do not indicate the wide range of seasonal and locational variability which occurs. The study by Howell and Collins (5) showed that environmental variability, particularly differences in temperature at the time of seed development, caused major differences in linolenic and linoleic acid contents. In fact, differences associated with temperature were of greater magnitude than those associated with varieties.

To provide more complete information on the variability in fatty acid composition of soybean oil, the present study was done on seed produced in 1956 and 1957 at 43 locations in 18 states.

Experimental

Soybean seed from Uniform Test Groups I, III, IV, and VII of the U. S. Regional Soybean Laboratory conducted at six to 14 locations were used in this study. Figure 1 (7) shows the approximate area of the United States where each Uniform Test Group is grown.

In 1956 oil was analyzed from the seeds produced by Groups I, III, and IV in each of four replications of each variety at each location and from three replications of Group VII. Analyses of the 1957 oil were made on duplicate samples of seed from selected locations in common with the 1956 tests. Oil was extracted by the official A.O.C.S. method (2), and immediately after extraction fatty acids in the crude oil were determined by the method of Collins and Sedgwick (4). Percentages of fatty acids are reported on a crude oil basis. The iodine values of the crude oil which were used in calculating percentages of oleic and saturated portions were obtained from the refractive index of the oil by the method of Majors and Milner (6).

The percentage values for saturated acids are higher than actual values because glycerol and some of the refining loss products in crude soybean oil were classed as saturated acids.

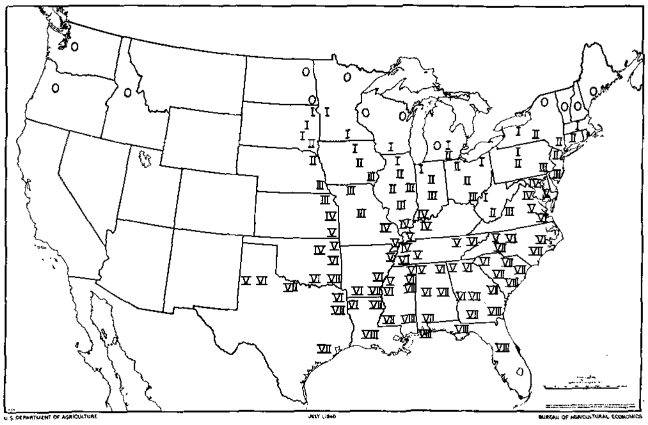

FIG. 1. Areas in the United States where soybean varieties in each of the maturity-classification groups are adapted as full-season crops.

Standard analysis of variance techniques of Snedecor (9) were used in the interpretation of the significance of the data.

Results

Data for the five varieties in Group I are summarized in Table I. Figure 2 shows the variability in composition of Chippewa soybean oil at 12 and nine locations in 1956 and 1957, respectively. Chippewa oil, in common with Blackhawk, Grant, and Mandarin (Ottawa), contained about 8.5% linolenic acid while Monroe had about 9.5%. Blackhawk tended to be lowest in both years in average percentage of linolenic acid (8.39%) at all locations, and Monroe averaged highest (9.40%). Chippewa oil was highest in the percentage of linoleic acid (50.8%).

Data for oils of six varieties in Group III are summarized in Table II. Figure 3 shows the variability in composition of Lincoln soybean oil for 11 locations in 1956 and eight in 1957. The mean percentages of linolenic and linoleic acids in Lincoln were similar for both crop years. In 1956 for Lincoln the ranges (not shown in the table) of linolenic, 7.70% to 8.98%, and linoleic, 49.1% to 52.9%, were similar to those for Chippewa in Group I, which had linolenic 7.75% to 9.62%, and linoleic 48.6% to 52.8%. Clark, Lincoln, and Shelby, three closely related varieties, con-

[1] Publication No. 326 of the U. S. Regional Soybean Laboratory, Urbana, Ill.