

## REFERENCES

1. McKay, D. C., and T. C. Wong, *J. Exp. Med.* **115**, 1117 (1962).
2. Raulin, J., C. Richir, L. Escriband and R. Jacquot, *Compt. Rend. Acad. Sci.* **248**, 1229 (1959).
3. Kaunitz, H., C. A. Slanetz, R. E. Johnson, H. B. Knight, D. H. Saunders and D. Swern, *JAOCS* **33**, 630 (1956).
4. Bloor, W. R., K. F. Pelkan and D. M. Allen, *J. Biol. Chem.* **52**, 191 (1922).
5. Sperry, W. M., and W. Webb, *J. Biol. Chem.* **187**, 97 (1950).
6. Folch, J., M. Lees and G. H. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957).
7. Kaunitz, H., C. A. Slanetz, R. E. Johnson, H. B. Knight, D. H. Saunders and D. Swern, *Fed. Proc.* **14**, 408 (1955).
8. Kaunitz, H., C. A. Slanetz and R. E. Johnson, *J. Nutr.* **62**, 551 (1957).

## Nutritive Value of Heated Vegetable Oils

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### Abstract

Peanut, sesame and coconut oils were heated at 270C for 8 hr, in an open iron pan. These fats were fed to albino rats at 15% level in otherwise adequate diets. All rats fed heated fats showed a growth depression. Livers of rats receiving heated oil were congested and showed extensive periportal fatty infiltration. Rats on heated peanut oil showed i) reduced B-vitamin storage in the liver, ii) increased glucose and cholesterol levels in the blood and iii) a disruption in the digestion and absorption of carbohydrates.

### Introduction

HEATED OILS have been shown to be poorly absorbed (13), to produce cancerous tumors (11) and to cause symptoms resembling that due to vitamin E deficiency (9). Lower nutritive value is also believed to be due to the destruction of certain vitamins, especially vitamin A (6). Further, it has been shown that the oxidation products of fats produced inhibitory effect on certain enzyme systems (4,12).

Consumption of fried foods in India is probably higher than in any other country. The conditions of heating are known to vary widely. The oils are usually heated in open air pans to about 200–300C depending on the type of preparation and the heated oils are reused.

The present investigations were carried out to evaluate the effect of heat on some edible oils commonly used in the country.

### Experimental and Results

#### Preparation of Heated Oils

Peanut, sesame and coconut oils were heated continuously at 270C for 8 hr in an open pan made of iron. The heated oils were stored in pyrex glass bottles at 0C.

#### Influence of Heating the Oil on Its Nutritive Value and Fat Deposition in the Liver

Thirty-six Wistar strain albino rats, five weeks old and weighing about 40–50 g from our laboratory stock colony were allotted to six groups in a randomized block design and were housed in individual cages. They were fed *ad lib.* on purified diets (fat, 15%; casein, 12%; sugar, 10%; salt mixture, 4%; vitaminized starch, 1% and corn starch, 58%) in which the fat was supplied by raw or heated peanut, sesame or coconut oils. At the end of six weeks feeding, the animals were killed by bleeding through the abdominal aorta. Livers, spleens, stomachs and kidneys were removed and weighed. The liver lipids were estimated by the method of Hawk (7). The results are presented in Table I.

The results show clearly that in all the three cases the heated oil has adversely affected the gain in weight. The feed efficiency ratio, calculated as increase in weight per gram of fat consumed, is significantly lower in groups receiving the heated oils. The livers of rats receiving the heated oil are significantly heavier than the controls. The lipid content of the livers is nearly twice that of the control groups. The livers showed signs of congestion and extensive periportal fatty infiltration. No significant changes were found in the weights of stomach, kidney and spleen of the two groups of animals.

#### Influence of Heated Oils on the Levels of B-Vitamins in the Livers

Eighteen male weanling albino rats, about 4 weeks old, were distributed into three groups in a random block design and were depleted of their B-complex stores by feeding a deficient diet for a period of two weeks. One group was sacrificed at this stage for determining the basal stores of B-vitamins in the liver which were as follows: thiamine, 11.7  $\mu$ g; riboflavin, 44.8  $\mu$ g; niacin, 285  $\mu$ g; pantothenate, 185  $\mu$ g and pyridoxine, 12.5  $\mu$ g. The two other groups were fed on a purified diet containing 15% of raw or heated peanut oil for a period of two weeks, after which

TABLE I  
Influence of Heating the Oil on Its Nutritive Value and Fat Deposition in the Liver

Dietary fat	Body weight		Mean gain per week (g)	Mean daily food intake (g)	Feed efficiency <sup>a</sup>	Liver weight		Liver fat %
	Initial g	Final (g)				(g)	g/100 g body weight	
Peanut oil:								
Raw	42.5	120.7	13.0 $\pm$ 0.5 <sup>b</sup>	11.3	1.1	4.58	3.8 $\pm$ 0.3	3.9 $\pm$ 0.3
Heated	42.0	72.1	5.0 $\pm$ 0.9	7.1	0.67	4.11	5.7 $\pm$ 0.6	7.1 $\pm$ 0.4
Sesame oil:								
Raw	42.5	102.5	10.0 $\pm$ 0.8	9.5	1.0	4.30	4.2 $\pm$ 0.7	4.8 $\pm$ 0.3
Heated	42.4	68.3	4.3 $\pm$ 0.7	5.9	0.7	4.10	6.0 $\pm$ 0.5	7.8 $\pm$ 0.3
Coconut oil:								
Raw	43.1	109.2	11.0 $\pm$ 1.1	9.5	1.1	4.70	4.3 $\pm$ 0.3	4.5 $\pm$ 0.3
Heated	42.8	69.9	4.5 $\pm$ 0.7	6.1	0.7	4.05	5.8 $\pm$ 0.4	7.0 $\pm$ 0.3

<sup>a</sup> Increase in weight per gram of fat intake.

<sup>b</sup> Standard error of the mean.

TABLE II  
Increase in the Liver Storage of B-Vitamins  
( $\mu\text{g}/\text{liver}$ )

Vitamin	Diet	
	Raw peanut oil	Heated peanut oil
Thiamine	25.6 $\pm$ 2.3 <sup>a</sup>	12.3 $\pm$ 1.6
Riboflavin	96.4 $\pm$ 8.6	67.4 $\pm$ 7.5
Niacin	652.0 $\pm$ 35.4	590.0 $\pm$ 34.6
Pantothenate	322.0 $\pm$ 21.6	175.0 $\pm$ 18.7
Pyridoxine	5.8 $\pm$ 0.3	2.5 $\pm$ 0.1

<sup>a</sup> Standard error of the mean.

they were sacrificed and the livers analyzed for the different B-vitamins. Thiamine, riboflavin and niacin were estimated according to the standard methods of the American Association of Vitamin Chemists (2). Pyridoxine and pantothenate were estimated microbiologically according to Barton-Wright (3).

It is evident from the results (Table II) that the liver storage of the different B-vitamins is adversely affected in the case of rats receiving the heated oil. This may be partly due to the lower food intake. It should be noted, however, that the different B-vitamins intake even by the rats receiving the heated oil diet is more than adequate.

**Effect of Heated Oils on the Absorption of Carbohydrates**

Two groups of thirty growing rats, weighing 120–130 g were fed purified diets containing 15% of raw or heated peanut oil for a period of 3 weeks. All the animals were fasted for a period of 24 hr. At this stage six animals in each group were sacrificed, and the total carbohydrate in the intestinal and stomach washings was determined. In the case of the fasted animals, the carbohydrate content in the intestinal and stomach washings was practically negligible. The remaining rats were fed orally a mixed diet at 1 g/100 g body weight of the rat, consisting of 120 mg casein, 150 mg oil, raw or heated, 690 mg of starch (includes 10 mg given as vitaminized starch) and 40 mg salt mixture. Six rats in each group were killed at intervals of 2, 3, 4 and 5 hr, and the intestinal and stomach washings were analyzed for starch and sugars according to the official methods of the Association of Agricultural Chemists (1). From the data, the percentage digestion and absorption of carbohydrates at different periods after feeding was calculated according to the following formulae:

$$\text{Digestion \%} = \frac{\text{Intake of starch} - \text{Amount of starch in the stomach and intestinal washings}}{\text{Intake of starch}} \times 100$$

$$\text{Absorption \%} = \frac{\text{Intake of starch} - (\text{Amount of starch in the stomach and intestinal washing} + \text{starch equivalent to the sugars present})}{\text{Intake of starch}} \times 100$$

The results given in Table III bring out clearly that digestion and absorption of carbohydrates are adversely affected by the presence of heated oil in the diet.

**Influence of Heated Oils on the Blood Sugar and Cholesterol Levels**

Two groups of six weanling rats weighing about 40–50 g were fed on purified diets containing 15% of raw or heated peanut oil for a period of 4 weeks. The animals were anaesthetized with ether and the blood was removed by heart puncture. Glucose and cholesterol in the whole blood were estimated by methods described by King and Wootton (10). The results are given in Table IV.

The results show that the presence of heated oil

TABLE III  
Digestion and Absorption of Carbohydrates in Rats  
Receiving Raw or Heated Peanut Oil Diets  
(Values are mean of 6 male rats in each group)

Time in hours after feeding	Raw peanut oil diet		Heated peanut oil diet	
	Digestion %	Absorption %	Digestion %	Absorption %
2	11.0 $\pm$ 0.6	7.0 $\pm$ 0.3	9.0 $\pm$ 0.5	4.0 $\pm$ 0.2
3	34.0 $\pm$ 5.6	21.0 $\pm$ 4.4	22.0 $\pm$ 4.3	13.0 $\pm$ 1.2
4	62.0 $\pm$ 8.7	49.0 $\pm$ 7.6	39.0 $\pm$ 3.5	23.0 $\pm$ 2.6
5	78.0 $\pm$ 6.4	68.0 $\pm$ 5.6	58.0 $\pm$ 5.4	35.0 $\pm$ 3.5

in the diet has been responsible for higher blood glucose and cholesterol levels.

**Discussion**

Present results indicate that heated oils have a growth-depressing action. The mechanism of this action is not clearly understood. Some workers (5) have attempted to correlate growth-depressing action of heated oils to polymer formation, but as no reliable method is available for determining the percentage of polymers in heated oils, this relationship is difficult to determine. Johnson et al. (8) observed that there was a rapid recovery of albino rats which had been changed from a thermally oxidized corn oil diet to a fresh corn oil diet, and this would seem to indicate that the thermally oxidized oil did not cause permanent metabolic damage. Some of our other investigations (in progress) have shown that the growth-depressing effect of heated oils could be counteracted by increased intake of sulfur amino acids. The diet used in the present study is essentially a low protein diet, and the effect of the sulfur amino acids may be due to the fact that these are the limiting amino acids in a purified 10% casein diet. The growth-depressing effect might also be, to a certain extent, due to the destruction of vitamins in the diet (6) or to a lowering in the activity of some enzymes (4,12).

The present studies have shown that there are large differences in the liver stores of B-complex vitamins of rats receiving raw and heated oil rations. These large differences cannot be entirely due to the destruction of the vitamins by the heated oil; faulty absorption of the vitamins is a point to be elucidated. The decreased digestion and absorption of carbohydrates in the case of rats receiving the heated oil may be due to lowered enzyme activity. The growth-depressing action of the heated oil may be due to the combined action of all the different factors and there is a necessity for further work to elucidate the correct mechanism.

Another significant observation, in the present study appears to be the effect of the heated oil in increasing the blood cholesterol of rats. The studies so far carried out on the effect of fats on cholesterol metabolism have been restricted to fats in raw form. Effect of heated fats on blood cholesterol merits a further study.

TABLE IV  
Blood Glucose and Cholesterol Levels of Rats  
Receiving Raw or Heated Peanut Oil Diets

Constituent ml/100 ml blood	Raw peanut oil	Heated peanut oil
Glucose	105 $\pm$ 5.6 <sup>a</sup>	131 $\pm$ 7.7
Cholesterol	76 $\pm$ 6.8	107 $\pm$ 3.8

<sup>a</sup> Standard error of the mean. All values are averages of 6 male rats in each group.

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## REFERENCES

1. Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," A.O.A.C., Washington, 8th Ed., 1955.
2. Association of Vitamin Chemists, "Methods of Vitamin Assay," Interscience Publishers Inc., New York, 1947.
3. Barton-Wright, E. C., "The Microbiological Assay of Vitamin B-Complex and Amino Acids," Sir Issac Pitman & Son Ltd., London, 1952.
4. Bernheim, F., K. M. Wilbur and C. B. Kenston, Arch. Biochem. Biophys. 33, 177 (1952).
5. Crampton, E. W., R. H. Common, F. A. Farmer, F. M. Berryhill and L. J. Wiseblatt, J. Nutr. 44, 177 (1951).
6. Dyme, H. C., Iowa State College J. Science 14, 29 (1939).
7. Hawk, E. A., and C. A. Elvehjem, J. Nutr. 49, 495 (1953).
8. Johnson, O. C., T. Sakuragi and F. A. Kummerow, JAOCS 33, 433 (1956).
9. Kaunitz, H., Arch. Exp. Path. Pharmacol. 220, 16 (1953).
10. King, E. J., and I. D. P. Wootton, "Micro-analysis in Medical Biochemistry," J. & A. Churchill Ltd., London, 1956.
11. Morris, H. P., and C. D. Larson, J. Nat. Cancer Inst. 4, 285 (1943).
12. Ottolenghi, A., F. Bernheim and K. M. Wilbur, Arch. Biochem. Biophys. 56, 157 (1955).
13. Ray, A., Ann. Biochem. Exp. Med. (India) 4, 17 (1944).

## Comparative Study of Monocarbonyl Compounds Formed During Deep Frying in Different Fats<sup>1</sup>

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### Abstract

Fresh commercial corn oil, fresh commercial lard, and hydrogenated vegetable shortening were analyzed for carbonyl compounds before and after deep frying. The frying was carried out in an apparatus with a capacity for 2000 g of oil designed to quantitatively trap the volatile materials evolved during frying and which would ordinarily escape into the atmosphere. The trapped distillate was also subjected to carbonyl analysis.

Analysis of the fats and distillates showed a carbonyl pattern in essential agreement with the classical autoxidation mechanism for the different fats, i.e., the typical alkanals, alk-2-enals, and alk-2,4-dienals. The pattern correlated generally with the fatty acid composition of the fats. Comparison of the concentrations of the monocarbonyl compounds in the fats before and after frying, and in their distillates, indicated that the deodorization process which accompanies frying is effective in preventing the accumulation of the more volatile compounds formed. The less volatile products, mainly deca-2,4-dienal, were not efficiently removed. Accordingly, it was observed that the oils containing higher proportions of linoleic acid contained more residual monocarbonyl compounds after frying.

CONSIDERABLE INTEREST has been stimulated by the possibility of toxic chemical alterations in fats during cooking. Thermal oxidation of unsaturated fats at 200C for 24 hr has been reported to produce substances toxic to rats (1). The efficacy of extrapolating from extreme laboratory conditions of heating and oxidation to the milder conditions of practical cooking has been vigorously questioned by Melnick (2,3,4) and Kaunitz (5). With the exception of the surveys made in the potato chip industry by Melnick, (2,3,4), there have been no definitive chemical studies of actual frying operations. The present work was designed to study the production of carbonyl compounds during the frying of potatoes under controlled laboratory conditions which would simulate good cooking practice. Carbonyl compounds were

chosen for study because of the availability of reliable analytical methods and the belief that as an important class of oxidation products, their study could provide information on the extent and type of oxidation. Knowledge of this type is necessary to help settle the controversy over the biological significance of heated fats.

### Experimental

**Potato Frying Operation.** In order to quantitatively trap the vapor evolved during the frying process, the apparatus illustrated in Figure 1 was designed. Vessel No. 1 was a 3 liter resin kettle adapted to a still-head. The kettle was heated with an electric mantle connected to a variable transformer. The opening at B was vented to a nitrogen source. Instead of a thermometer at D, a stainless steel wire entering the apparatus around a rubber stopper was used to suspend the stainless steel basket containing the potatoes. This basket could be lowered and raised without removing the stopper. The distillate was collected in three low-temperature traps 2, 3, and 4. Trap No. 2 (2 liters) was mounted under a cold-water condenser, and was cooled by an ice-water bath. The major portion of the distillate was retained at this point. Traps 3 and 4 were mounted under cold-finger condensers charged with an ethanol-dry ice mixture. They were also cooled by ice-water baths. Trap No 5, containing 300 ml of 3 M phosphoric acid saturated with 2,4-dinitrophenylhydrazine, was used only when the system was under

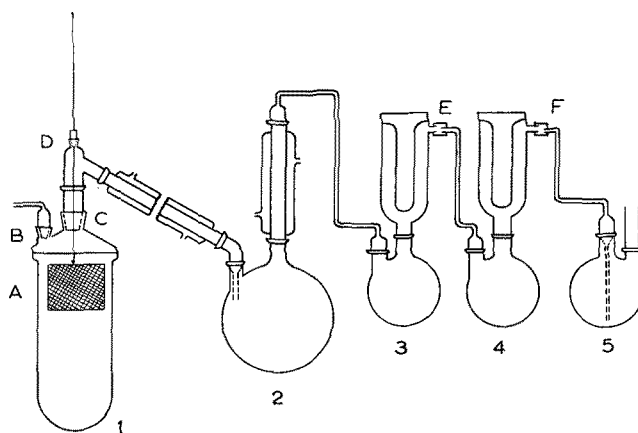


FIG. 1

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