Toxicological and nutritional evaluation of five different heated oils in rats

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Abstract

Five different vegetable oils, viz. peanut oil (PO), sunflower oil (SFO), soybean oil (SBO), low-erucic-acid rapeseed oil (LEAR) and a mixture of palm oil, sunflower oil and rapeseed oil (PSRO; C 18:3 < 2.0 %) were subjected to a heat-treatment comparable to that of deep-fat frying. The oils were heated on 20 consecutive days. The temperature was raised to 220 °C and kept above 200 °C for about 12 hours in total. The oils subjected to 20 heating cycles and the non-heated oils were fed to rats at a dietary level of 15 % for a period of 19 weeks. After 10 weeks the rats were mated within each diet group in order to examine possible effects of the heated oils on reproduction.

The results of the chemical characterization of the heated and non-heated oils indicate that PO and LEAR were the most stable ones towards heat-treatment.

No outstanding differences in growth, food intake, food efficiency, plasma transaminase activity, plasma cholesterol, retinol, lipoprotein content and microscopy of several organs were observed between the heated and non-heated oil groups. Fat digestibility was slightly lower and plasma α-tocopherol levels were considerably lower in the heated oil groups than in the non-heated controls. There was an increase in the relative weight of the liver in females fed heated PO, heated SFO or heated PSRO. Relatively high liver weights were also observed in the groups fed LEAR, both heated and non-heated.

The reproduction study did not reveal any adverse effects of any of the heated oils on fertility, litter size at birth or mortality of the pups. Body weight gain of the pups during lactation was significantly depressed in all groups fed heated oil, except in the group fed heated PO.

In general, the results obtained with LEAR were not distinguishable from those obtained with the other oils.

INTRODUCTION

In deep-fat frying, often the fat or oil is kept hot for long periods at temperatures which may exceed 200 °C. Heating causes measurable changes in the chemical and physical characteristics. These changes include the formation of low molecular oxidation products such as peroxides, carbonyls etc. or high molecular weight compounds such as dimeric- and polymeric triglycerides, which increase the viscosity of

the frying fat. Many reports of experimental studies with animals show that the biological properties of heated fats or oils are closely related to their chemical properties. Growth retardation, reduced food efficiency, decreased haemoglobin content of the blood, increased liver size and microscopical abnormalities of the liver are common findings upon feeding heated oil.

In many countries, for instance in France, especially oils are used for frying. Since oils are more susceptible to the damaging effect of heat-treatment than fats - because of the higher content of unsaturated fatty acids - the health risk involved in the ingestion of products fried in oils might be correspondingly higher than that associated with consuming products fried in fats or hydrogenated oils.

Some countries have attempted to regulate to what degree an oil or fat is allowed to be deteriorated by heating before it should be condemned. In The Netherlands, the content of polymeric triglycerides should not exceed 10 per cent (Van der Heide, R.F. and J. van der Veen, 1977). In the Federal Republic of Germany a level of 27 per cent of polar compounds is considered the upper limit (Billek, G. et al., 1979). In France, there is an ordinance of 1973 saying that oils with a linolenic acid level higher than 2 per cent should not be used for deep-fat frying.

In low-erucic-acid rapeseed oil usually a linolenic acid content of approximately 9 per cent is found and should, therefore, like soybean oil with about 6 per cent linolenic acid, not be used for frying. In order to obtain more information on the effect of heat-treatment on the chemical and biological properties of a commercial low-erucic-acid French rapeseed oil (LEAR), we undertook a study in which heated and non-heated LEAR was fed to rats at a dietary level of 15 per cent for 19 weeks. Heated and non-heated peanut oil (PO), sunflower oil (SFO), soybean oil (SBO) and a mixture of palm oil, sunflower oil and low-erucic-acid rapeseed oil (51/29/20; PSRO) were tested simultaneously for comparative purposes. The study was sponsored by CETIOM, Paris, France, under the supervision of CNERNA, Paris, France.

HEAT-TREATMENT AND CHEMICAL ANALYSES OF THE OILS

The fatty acid composition of the five non-heated oils, determined by capillary GLC, is given in table 1.

TABLE 1 - FATTY ACID COMPOSITION OF THE NON-HEATED OILS IN WEIGHT % OF THE FATTY ACID METHYL ESTERS

Fatty acid	peanut oil	sunflower oil	soybean oil	rapeseed oil	palm/ sunflower/ rapeseed
12:0	-	-	-	-	0.2
14:0	-	-	0.1	-	0.5
16:0	10.7	5.7	10.7	5.2	21.7
16:1	0.1	-	0.1	0.3	0.1
17:0	0.1	-	0.1	-	0.1
18:0	3.4	4.5	4.0	1.6	4.2
18:1	46.9	16.6	23.9	57.3	38.2
18:2	31.7	71.5	53.3	21.8	31.7
18:3	0.1	0.1	6.0	8.7	1.8
20:0	1.5	0.3	0.4	0.6	0.4
20:1	1.1	0.1	0.2	1.8	0.5
22:0	3.1	0.7	0.4	0.2	0.2
22:1	-	-	-	1.9	0.3
24:0	1.3	-	-	-	-

The following remarks can be made: The linoleic acid content of PSRO was similar to that of PO, but differs in that it has a higher linolenic acid content of 1.8 %, which is, however, lower than the highest permittable level of 2 % mentioned in the French ordinance. The rapeseed oil was low in erucic acid (1.9 %) and high in oleic acid (57.3 %). Relatively high levels of linolenic acid were found both in SBO and LEAR.

30 l of each type of oil were heated simultaneously in deep fat fryers (type: Becuwe Thomselle) on 20 consecutive days. Every day the following temperature cycle was applied: Ambient - 220 °C - 200 °C - 220 °C - ambient. In total (20 days) the temperature was kept above 200 °C for about 12 hours. No foods were fried. To follow the degree of deterioration of the oil, samples were taken after 0, 5, 10, 15 and 20 days of heat-treatment and analyzed for a.o. polar material (Guhr, G. and J. Waibel, 1978) and polymeric triglycerides (by High Pressure Gelpermeation Chromatography). These two parameters can be considered as good indicators of the heat-damage of the oils.

The results obtained are given in figures 1 and 2.

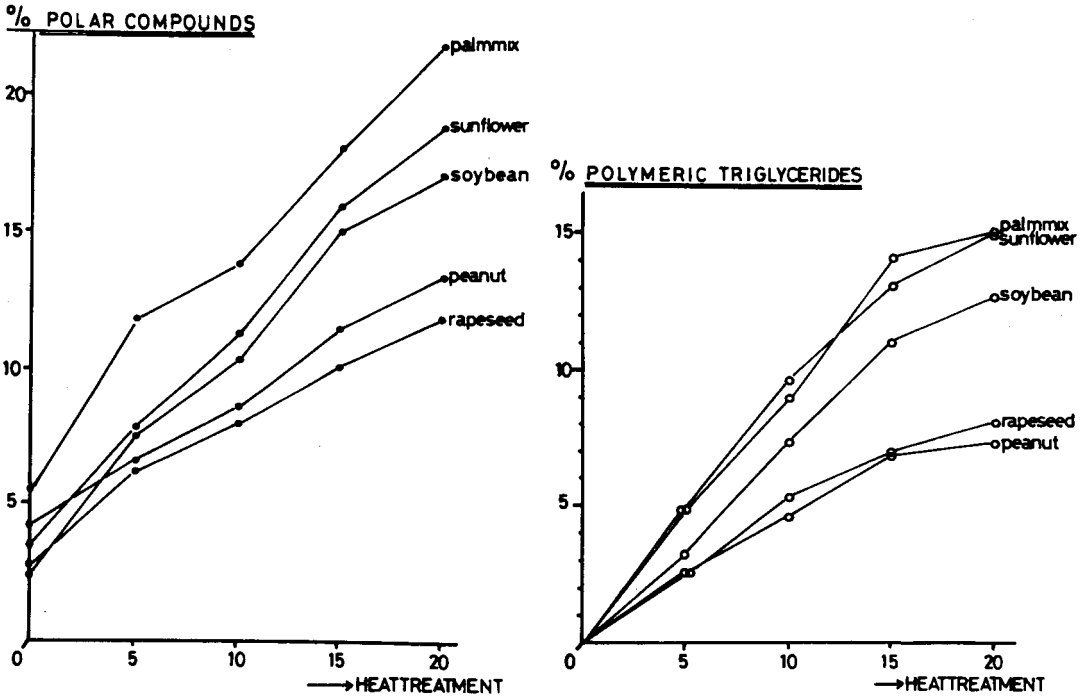

Fig. 1 and 2 - EFFECT OF HEAT-TREATMENT ON THE PERCENTAGE OF POLAR COMPOUNDS AND POLYMERIC TRIGLYCERIDES OF FIVE DIFFERENT OILS

The contents of polar material and polymeric triglycerides showed a gradual increase with an increasing number of heat-treatments. The highest levels after 20 days of heat-treatment were observed with PSRO, SFO and SBO, whereas PO and LEAR showed the lowest levels, indicating that the latter two oils were the most stable ones towards heat-treatment.

FEEDING STUDY IN RATS

The oils obtained after 20 heating cycles were used for a combined subchronic toxicity and reproduction study in rats. For that purpose, the oils, both heated and non-heated, were incorporated in a, nutritionally well-balanced, semi-purified diet at a level of 15 % and fed to groups of 20 male and 20 female Wistar rats (Cpb:WU) for a period of 19 weeks. For the composition of the diets, see table 2.

TABLE 2 - COMPOSITION OF THE DIETS

Ingredient	Weight %
Casein	22
Dl-methionine	0.2
Wheat starch	26.5
Saccharose	26.5
Cellulose	5
Vitamin ADEK mixture[1)]	0.35
Vitamin B mixture[2)]	0.25
Mineral mixture[3)]	4.20
Heated or non-heated oil	15
Total	100.00

1) Per one g mixture: vitamin A 2215 IU, vitamin D_3 705 IU, vitamin E 15 mg, vitamin K_3 1 mg.

2) Per one g mixture: vitamin B_1 3 mg, vitamin B_2 2.25 mg, vitamin B_6 4.5 mg, niacin 15 mg, Ca-panthothenate 6 mg, biotin 0.075 mg, folic acid 0.75 mg, vitamin B_{12} (0.1 %) 37.5 mg, choline chloride (50 %) 931 mg.

3) Per one g mixture: KH_2PO_4 399 mg, $CaCO_3$ 389 mg, NaCl 142 mg, $MgSO_4$ 58 mg, $FeSO_4.7\ H_2O$ 5.7 mg, $ZnCl_2$ 0.9 mg, $CuSO_4.5\ H_2O$ 0.8 mg, $MnSO_4.2\ H_2O$ 4.6 mg, $CoCl_2.6\ H_2O$ 0.02 mg, KJ 0.07 mg, $KCr(SO_4)_2.12\ H_2O$ 0.08 mg.

The rats were housed under conventional conditions, five per sex per cage, in suspended stainless steel cages, fitted with wire mesh floors and fronts, in a room which was controlled with respect to ventilation (c. 10 air changes per hour), temperature (23 $\pm$ 1 °C) and relative humidity (50 $\pm$ 10 %). A 12-hour light/dark cycle was maintained.

At week 10 of the study, males and females within each group were allowed to mate, one male to one female, for a period of three weeks, in order to examine possible effects of the heated oils on reproduction.

Observations were made of behaviour and with respect to general health. In the parent animals the following determinations were carried out:

body weight, food intake and food efficiency: weekly during the first 10 weeks of the study; digestibility of fat and protein: week 10; in terminal blood samples: GOT and GPT (Lippi and Guidi-method), vitamin A and E (high speed liquid chromatography and HPLC respectively), cholesterol and lipoproteins (enzymatic method and electrophoresis on agarose gel). After 19 weeks of feeding the various diets, the parent rats were killed, the liver, heart, kidneys, thymus, adrenals and gonads were weighed and subjected to microscopical examination.

In the reproduction study observations were made as well as calculations of the fertility index of the females, the mean litter size at birth, the mean pup weight at birth and on day 4, 14 and 21 after birth, pup mortality at birth and during lactation. From the number of implantation sites found in the uterus of the mothers and the total number of pups born, the resorption quotient was calculated as the total number of implantations/total number of pups born.

Most of the data were analyzed statistically by a two-way analysis of (co)variance, whereby the effect of heating, type of oil and interaction between heating and type of oil were examined.

RESULTS

Data on body weight gain, food consumption and food efficiency are summarized in table 3.

TABLE 3 - BODY WEIGHT GAIN, FOOD INTAKE AND FOOD EFFICIENCY. MEAN VALUES OF GROUPS OF 20 RATS

Diet group	Body weight gain (g/rat) week 0-10	Food intake (g/rat) week 0-10	Food efficiency (gain/food) week 0-10	Body weight gain (g/rat) week 0-10	Food intake (g/rat) week 0-10	Food efficiency (gain/food) week 0-10
	MALES			FEMALES		
PO-control	274.7	1030	0.267	136.6	739	0.185
PO-heated	291.3	1086	0.268	136.3	738	0.185
SFO-control	283.1	1031	0.275	128.7	717	0.179
SFO-heated	277.3	1045	0.265	135.2	714	0.189
SBO-control	292.4	1052	0.278	132.9	700	0.190
SBO-heated	286.4	1023	0.280	142.0	741	0.192
LEAR-control	266.4	1017	0.262	128.2	705	0.182
LEAR-heated	264.9	1015	0.261	136.9	720	0.190
PSRO-control	286.4	1045	0.274	133.6	704	0.190
PSRO-heated	266.7	1010	0.264	136.4	732	0.186

There were no outstanding differences in body weight gain amongst the various groups. Only in males fed LEAR, both heated and non-heated, body weight gain was less than in the other groups. The differences with the other groups were statistically significant in most of the weeks examined. Food intake figures were generally comparable amongst the groups. Males fed heated SBO or heated PSRO tended to eat less than the corresponding controls, but in females the opposite phenomenon occurred. Food efficiency was slightly lower in males fed LEAR, both heated and non-heated, but no such an effect was noticeable in females.

The results of determinations of the digestibility of fat and protein are given in table 4.

TABLE 4 - DIGESTIBILITY OF FAT AND PROTEIN DETERMINED DURING WEEK 10 OF THE STUDY

Diet group	Males		Females	
	Digestibility coefficient (%) for		Digestibility coefficient (%) for	
	Fat	Protein	Fat	Protein
PO-control	93.5	78.2	95.9	84.8
PO-heated	93.1	81.3	94.9	75.4
SFO-control	96.0	79.2	97.6	83.7
SFO-heated	94.1	76.2	94.7	82.8
SBO-control	96.1	74.4	97.7	84.6
SBO-heated	95.0	80.7	97.3	85.8
LEAR-control	94.8	82.6	96.8	87.4
LEAR-heated	94.4	78.2	95.7	89.0
PSRO-control	95.3	79.1	97.0	86.0
PSRO-heated	93.8	78.6	94.2	84.3

The digestibility of fat and protein was high in all groups. However, the digestibility of fat in the diets with heated oil was consistently slightly lower than that of the corresponding diet with non-heated oil. The differences were greatest with the oils containing the highest content of polymeric triglycerides viz. heated SFO and heated PSRO. The digestibility of protein was not noticeably affected by the heat treatment of any of the oils.

The levels of vitamin A and E determined in pooled plasma samples at the end of the study are presented in table 5, together with the content of vitamin A and E in the diets.

TABLE 5 - RESULTS OF VITAMIN A AND E DETERMINATIONS IN FOOD AND PLASMA OF RATS

Diet group	Vitamin A in diet (IU/g)	Retinol content (µ mol/l) of plasma[1]		α-tocopheryl acetate in diet (µg/g)	α-tocopherol content (µ mol/l) of plasma[1]	
		males	females		males	females
PO-control	12.9	1.50	0.80	85	33.4	31.4
PO-heated	12.8	1.45	0.83	65	22.4**	23.9**
SFO-control	12.8	1.35	0.85	160	35.4	30.7
SFO-heated	12.8	1.28	0.98	100	21.3***	20.6**
SBO-control	14.0	1.40	0.85	75	31.2	30.0
SBO-heated	13.2	1.28**	0.93	70	18.8***	22.7*
LEAR-control	12.8	1.48	0.68	110	36.9	31.6
LEAR-heated	13.0	1.50	0.73	80	22.1**	22.4***
PSRO-control	13.0	1.20	0.60	110	38.3	32.8
PSRO-heated	13.0	1.18	0.70*	70	17.6*	20.6**

1) Values are the mean of 4 pooled samples of 5 rats/sex/group.

Degrees of significance (Student t test): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The plasma levels of vitamin E (α-tocopherol) of the groups fed the heated oils were considerably lower than those of the controls fed the corresponding non-heated oils, both in males and females. It is most likely that the decreased plasma levels are due to the lower vitamin E contents of the diets containing heated oil as compared with the corresponding diets containing non-heated oil. The relatively low α-tocopherol levels of the diets with heated oil can probably be accounted for by losses of vitamin E, naturally present in the oils, during the heat-treatment. The vitamin A levels of both the diets and the plasma samples were rather uniform amongst the groups.

The determinations of cholesterol, lipoproteins, glutamic-oxalacetic transaminase- and glutamic-pyruvic transaminase activity did not reveal any outstanding differences amongst the groups.

The mean relative weight of the liver, determined at autopsy after a 19-week feeding period, is given in table 6.

TABLE 6 - MEAN RELATIVE LIVER WEIGHT OF GROUPS OF 20 MALE AND 20 FEMALE RATS AFTER FEEDING THE VARIOUS DIETS FOR 19 WEEKS

Diet group	Mean relative liver weight (g/kg) ± SEM	
	Males	Females
PO-control	29.3 ± 0.5	32.6 ± 0.6
PO-heated	29.3 ± 0.6	35.1 ± 0.9
SFO-control	30.5 ± 0.5	31.6 ± 0.6
SFO-heated	29.9 ± 0.4	35.0 ± 0.8
SBO-control	30.1 ± 0.6	34.5 ± 0.5
SBO-heated	30.0 ± 0.6	33.3 ± 0.6
LEAR-control	32.4 ± 0.5	35.6 ± 0.7
LEAR-heated	32.9 ± 0.4	35.1 ± 0.5
PSRO-control	30.5 ± 0.5	32.7 ± 0.6
PSRO-heated	31.6 ± 0.5	35.5 ± 0.7

There was no effect of heat-treatment on the relative weight of the liver in males. In females, however, there was a statistically significant increase in liver weight in the groups fed heated PO, SFO or PSRO. The values found in these groups (c. 35 g/kg) were of the same magnitude as those found in the groups fed heated or non-heated LEAR. Relatively high liver weights were also observed in males fed heated or non-heated LEAR.

Gross examination at autopsy and microscopical examination of kidneys, liver, heart, testes, adrenals, pancreas, ovaries and thymus did not reveal any abnormality attributable to the feeding of the heated or non-heated oils. The macroscopical as well as microscopical observations in the groups fed LEAR were not essentially different from those in the other groups.

In table 7 a summary of the reproduction data is given.

TABLE 7 - SUMMARY OF THE REPRODUCTION DATA

Diet group	Fertility[1] index (%)	Mean litter size at birth	Mean pup weight at birth (g)	Mean pup weight at day 21 (g)	Pup mortality at birth (%)	Pup mortality during lactation (%)	Resorption[2] quotient
PO-control	95	10.9	5.6	45.5	5.3	10.0	1.11
PO-heated	100	9.6	5.9	44.9	3.1	1.5	1.85
SFO-control	95	8.3	5.2	45.9	22.9	24.3	2.10
SFO-heated	89	10.2	5.1	38.1	21.3	21.8	1.33
SBO-control	100	11.4	5.5	44.7	9.7	16.0	1.10
SBO-heated	95	10.9	5.4	38.3	11.1	21.5	1.12
LEAR-control	100	10.9	5.2	43.1	4.1	18.5	1.13
LEAR-heated	100	11.6	5.5	38.2	1.3	6.3	1.09
PSRO-control	100	10.0	5.5	47.6	4.0	15.9	1.17
PSRO-heated	95	10.2	5.8	38.9	9.3	11.9	1.15

1) Fertility index = no. of pregnancies/no. of matings x 100 %.
2) Resorption quotient = total no. of implantations/total no. of pups born.

The number of successful matings, as expressed by the fertility index, was high in all groups and did not appear to be affected by the heat-treatment of any of the oils. Mean litter size at birth was relatively low in the group fed non-heated SFO. A consistent difference between the groups fed heated oil and the corresponding controls was, however, not apparent. Body weight gain of the pups during lactation was significantly depressed in all groups fed heat-treated oil, except in the group fed heated PO. The growth retardation was noticeable already on day 14 and was more marked on day 21, resulting in statistically significant differences between the heated and non-heated oil groups. In the groups fed SFO, both heated and non-heated, the mean pup weight at birth was decreased in a statistically significant way as compared with most other groups. Mortality of pups at birth and during lactation was extremely high in both SFO-fed groups. In the LEAR-fed groups mortality figures were not distinguishable from those of the other groups. The resorption quotient was unusually high in the group fed heated PO and especially in the group fed non-heated SFO. There was, however, a large variation amongst the resorption quotient of the individual animals in these groups. An effect of the heat-treatment of the oils on the resorption quotient was not apparent.

The groups fed heated LEAR showed the most favourable reproduction data: the highest number of pups at birth with a relatively high body weight, low mortality figures at birth and during lactation and the lowest resorption quotient.

DISCUSSION AND CONCLUSION

Feeding studies in rats conducted at our institute with oils and fats that had been submitted to severe heat-treatment revealed growth depression accompanied with decreased food intake and food efficiency, decreased haemoglobin content of the blood and increases of the relative weight of the liver and occasionally also of the kidneys (Van der Heide, R.F. and J. van der Veen, 1977). In the present study, the parent rats did not exhibit decreased growth rate, food intake or food efficiency in any of the groups fed heated oil. The heat-treatment applied was probably not sufficiently severe to induce the above mentioned changes.

The digestibility of the fat in the diets with heated oil was consistently slightly lower than that in the diets with the corresponding non-heated oil, both in males and females. The differences were greatest with the oils containing the highest content of polymeric triglycerides viz. heated SFO and heated PSRO. This relationship suggests that the decreased digestibility of the heated oil had possibly been caused by the presence of polymeric triglycerides.

The slightly increased liver weights observed in some groups of females fed heated oil were not accompanied with histopathological changes or with increases in transaminase activity, which usually indicate liver damage. The increased liver weights are therefore considered a reflection of altered metabolic processes in the liver, rather than of hepatotoxic properties of heated oils. The relatively high liver weight in the groups fed both heated and non-heated LEAR may likewise be accounted for by altered metabolic processes in the liver due to xenobiotic substances in LEAR.

The only effect on reproduction which can be related to the feeding of the heat-treated oils was a decrease in growth rate of the pups during the third week of lactation. This phenomenon was hardly noticeable in the group fed heated PO, but in the other groups fed heated oil, the mean pup weight was 11-18 % lower than in the corresponding group fed non-heated oil. Lower pup weights at day 21 were also observed in a comparable study of Coquet, B. et al. (1977), although the differences between the groups fed heated or non-heated oil were less pronounced than in the present study. The growth depression mainly occurred during the last week of lactation, when the pups are no longer solely dependent on breast milk, because they eat also from the feeders. In palatability tests it has been established that the rats showed a dislike for the diets with heated oil (Sinkeldam, E.J., unpublished observations). Therefore, a reduced food intake might have been the cause of the decreased growth rate.

The relatively high number of pups born dead in the groups fed heated or non-heated SFO was remarkable. In the group fed non-heated SFO this finding was accompanied with a relatively small litter size, a low pup weight at birth and a high number of resorptions in utero. A relatively high pup mortality with SFO has also been observed by Guillaumin, R. et al. (1980). The cause of the phenomenon with SFO remains unclear.

From the results obtained it seems justified to conclude that the feeding of the five heated oils to rats at a dietary level of 15 % for 19 weeks did not induce effects of obvious toxicological significance. With respect to the deteriorating effect of heat-treatment the two oils with a relatively high linolenic acid content, viz. LEAR and SBO, were not worse than the other oils. On the contrary LEAR was, with PO, the oil most stable towards heat-treatment.

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