Effect of Ingestion of Thermally Oxidized Frying Oil on Peroxidative Criteria in Rats

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ABSTRACT

Thermally oxidized rapeseed oils (4 levels of deterioration; used by a manufacturer of fried fish paste in a conventional manner) were fed to rats at a practical level of concentration. Rats were fed a diet ad libitum for 13 weeks that contained 15% of a test oil. The effects of the diet on several biochemical criteria related to peroxidative alterations were investigated.

In groups given thermally oxidized oils relative liver weight, relative kidney weight, thiobarbituric acid-reactive substances (TBA-RS) in the liver and reduced glutathione content were increased significantly in proportion to the degree of deterioration of the oil, compared with the group given fresh oil. Tocopherol contents in both serum and liver were decreased considerably in proportion to the deterioration level of the supplied oils.

The above criteria correlated well with various deterioration indices of the oil. For instance, TBA-RS was well correlated ($p<0.001$) with petroleum ether-insoluble oxidized fatty acid ($r=0.9191$), column chromatographically separated polar fraction ($r=0.9056$), glyceride dimer fraction ($r=0.9023$) and carbonyl value ($r=0.8647$).

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Because of the continued popularity of foods prepared by deep fat frying, establishing limitations on the extended usage of frying oils for both quality control and consumer protection has become important. Since Crampton et al. (1) first found toxic compounds in heated fats, some controversy has been generated regarding them. Reviews have been published by Nolen et al. (2), Mankel (3), Poling et al. (4), Waltking et al. (5) and Alexander (6). Broadly speaking, there are two schools of thought. One group of researchers (7,8) has isolated compounds from oils subjected to prolonged heating in the laboratory by vacuum distillation, urea adduct formation or column chromatography. They have observed pathological responses such as appetite and growth depression, diarrhea, histological changes in various tissues, and even death in some cases, after feeding these compounds to animals. This group takes a serious view regarding the potential toxicity of heated fats. The other group of researchers (2,4) has generally taken commercially used frying oils and either attempted to estimate the amount of the questionable components or fed the whole used fat to experimental animals as part of a balanced diet throughout the lifetime of the animals. The latter studies suggest that the suspected components are not present in ordinary commercially used oils in a significant quantity, and no deleterious effects were detected in the feeding studies.

To resolve the controversy, an experimental model that permits quantitative assessment of possible harmful effects of a heated fat was set up. For this purpose, we may use several biochemical criteria related to peroxidative alterations for detecting mild toxicity or low levels of toxic substances. According to Andia and Street (9), the ingestion of thermally oxidized oil increases endogeneous malonaldehyde. Peroxidative alterations have been identified as a basic deteriorative reaction (10) in the cellular mechanisms of aging and pathological disorders, and may be useful as a toxic response criterion at the biomembrane level.

In this study we attempted to define the influence of whole commercially used oils on the several biochemical criteria related to peroxidative deterioration, using rats fed a balanced diet containing the oils in a practical range of concentration.

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ABBREVIATIONS

ox.FA: Petroleum ether-insoluble oxidized fatty acid
CC-PF: Column chromatographically separated polar fraction
GD: Glyceride dimer fraction
TLC-UV_{233}: Thin-layer chromatography with ultraviolet detection at 233nm
P/Ap: Polar/apolar ratio measured by TLC-UV_{233}
RIT: Relative interface tension
TBA-RS: Thiobarbituric acid-reactive substance.

MATERIALS AND METHODS

Sample Oil Preparation

Thermally oxidized rapeseed oils were obtained from a manufacturer of fried fish paste (a traditional Japanese fried food named "Satsuma'age"). The fresh rapeseed oil (low erucic acid rapeseed oil containing citric acid at 20 ppm and silicone oil at 1.5 ppm, but not BHA or BHT; Yoshihara Seiyu Co., Ltd. Kobe, Japan) was used for frying fish

paste for a maximum of 66 days in an auto fryer (frying temperature, 180 C; heating time, 3.5 hr/day; capacity, 39.0 kg; surface area, 7,700 cm^2; fat turnover ratio, 4.1%/hr). Appropriate oil was added to the fryer at the end of each day to replace oil removed by sampling or adsorption by the fried fish paste.

Quality Assessment of Sample Oils

The quality of the frying oils was checked daily. Oils showing 4 levels of deterioration were used for animal feeding tests. The sample oils were kept in glass bottles under nitrogen at a temperature below 5 C. Carbonyl value (COV), peroxide value (POV), acid value (AV) and iodine value (IV) were measured by the standard methods of the Japanese Oil Chemists' Society (11). COV was represented as meq/kg for convenience in comparing it with other data. Petroleum ether-insoluble oxidized fatty acid (ox.FA) was determined by DGF-Einheitsmethoden C-III-3, rapid test (12). Column chromatographic separation of the polar fraction of frying oils has been proposed as a IUPAC-AOCS method (13). Glyceride dimer (GD) fraction, abundant in functional groups, was determined by silica gel column chromatography according to the method of Ohfuji and Kaneda (8). The polar/apolar (P/Ap) ratio was determined by the thin-layer chromatography with ultraviolet detection at 233nm (TLC-UV_{233}) method according to Urakami et al. (14). Relative interface tension (RIT) measurement has been proposed by Yoshikawa et al. (15) as a simple and convenient method to assess the quality of used frying oil. Tocopherols in oils were determined by a high-pressure liquid chromatography (HPLC) method with spectrofluorometric detection (16). The fatty acid composition of test oils were estimated by gas chromatography (GC) (11) using "Fat and oil reference mixtures No. 3" (Applied Science Lab., State College, PA) as a standard. C_{18}-Cyclic monomers were determined by GC according to the method of Meltzer et al. (17).

Animals and Treatments

Male COBS-Fisher (F-344/Ducrj) rats (Japan Charles River, Kanagawa, Japan), obtained at 4 weeks of age, were housed in individual wire cages. After one week for acclimation, rats weighing 98 to 108 g were randomly divided into 5 groups.

The basal diet [Oriental refined diet B with fat eliminated and selenium (0.5 ppm) added as sodium selenite] was purchased from Oriental Yeast Co. (Tokyo, Japan). The experimental diet also contained the selected amount (15%) of a test oil. The composition of the experimental diet is shown in Table 1.

The experimental diet was prepared once a week and stored in a closed container kept at a temperature below 5 C. Uneaten food was discarded every 3-4 days. Food and water were provided ad libitum, and food intake and body weight were measured every 3-4 days.

TABLE 1

Composition of Experimental Diet

Basal diet	
Cornstarch	34.4%
Milk casein	22.6%
Alpha starch	9.0%
Cellulose powder	7.2%
Granule sugar	4.5%
Vitamins[a]	1.8%
Minerals[b]	5.4%
Test oil	15.0%

[a]Vitamins (in 1 kg diet): retinyl acetate, 3.23 mg; cholecalciferol, 45.3 μg; thiamine HCl, 22 mg; riboflavine, 72 mg; pyridoxine HCl, 15 mg; cyanocobalamin, 90 μg; ascorbic acid, 540 mg; dl-α-tocopherol acetate, 90 mg; phylloquinone, 91 mg; biotin, 0.4 mg; folic acid, 3.6 mg; Ca-pantothenate, 90 mg; p-aminobenzoic acid, 90 mg; niacin, 90 mg; inositol, 109 mg; choline-Cl, 3.62 g.

[b]Minerals (in 1 kg diet): K, 4.01 g; P, 5.7 g; Ca, 4.31 g; Na, 1.97 g; Mg, 543 mg; Fe, 259 mg; Zn, 31 mg; Mn, 14 mg; Cu, 4 mg; I, 4 mg; Se, 0.5 mg.

Biological Procedures

At completion of the study (after 13 weeks of treatment with the experimental diet), the rats were killed by exsanguination under a light ether anesthesia. Blood was centrifuged at 2,500 rpm and thiobarbituric acid-reactive substance (TBA-RS) (18) and tocopherols (19) were estimated in the resultant serum.

The liver and kidneys were immediately excised, trimmed of connective tissues, and weighed. Samples of tissues (3 g) were weighed, added to 27 ml of cooled potassium phosphate buffer (75 mM, pH 7.0) and kept refrigerated. Homogenation and subcellular fractionation were done according to the method of Tsai et al. (20) as quickly as possible to avoid changes after excision. TBA-RS was determined by the method of Ohkawa et al. (21) and/or Uchiyama and Mihara (22), using 0.5 ml of 10% (w/v) whole homogenate, with tetraethoxypropane as a standard, and was expressed as malonaldehyde. Tissue-reduced glutathione (GSH) was determined by Ellman's method (23) with minor modifications.

After subcellular fractionation, the 100,000 g supernatant was assayed for glutathione peroxidase (GSH-px) and glutathione reductase (GR) activities. GSH-px activity was estimated by Little's method (24) using tertbutyl hydroperoxide as the substrate, which measures not only GSH-px activity but also GSH-S-transferases as well. GR activity was determined according to "Methods of Enzymatic Analysis" (25). Liver lipid was extracted with

TABLE 2

Characteristics of Test Oils

	Fresh	Heat-treated			
		A	B	C	D
Frying time (hr)	0	10.5	31.5	87.5	231.0
Carbonyl value (meq kg)	6.0	44.4	95.9	159.1	126.4
Peroxide value (meq kg)	1.1	5.0	8.9	7.6	5.3
Acid value	0.1	0.8	1.0	1.9	4.6
Iodine value	119.1	114.3	109.7	100.0	104.2
Polar material					
ox.FA (%)[a]	0.0	0.5	1.7	5.1	4.7
CC-PF (%)[b]	2.1	10.3	21.5	39.1	39.7
Glyceride dimer (%)[c]	0.0	0.6	1.0	2.8	2.3
P, Ap (TLC-UV$_{233}$)[d]	0.11	2.18	4.73	9.14	5.64
Viscosity (cp. 20 C)	99	117	155	270	281
Relative interface tension[e]	0.90	0.70	0.58	0.51	0.45
Tocopherols (μg g)	α.136.0				
	γ.367.5	0	0	0	0
	δ, 3.6				

[a]Petroleum ether-insoluble oxidized fatty acid.
[b]Polar fraction separated by silica gel column chromatography according to Waltking and Wessels (13).
[c]Glyceride dimer fraction obtained by silica gel column chromatography according to Ohfuji and Kaneda (8).
[d]Polar:apolar ratio measured by the TLC-UV method of Urakami et al. (14).

TABLE 3

Fatty Acid Composition of Test Oils[a]

	Fresh	Heat-treated			
		A	B	C	D
16:0	3.5	3.6	3.6	3.5	3.7
18:0	1.7	1.7	1.7	1.7	1.7
18:1	57.2	56.3	53.5	49.9	53.2
18:2	20.7	19.4	17.5	14.2	15.2
18:3	14.6	11.4	9.6	7.4	8.0
22:0	0.4	0.4	0.4	0.3	0.4
22:1	2.2	2.2	2.1	2.0	1.9
Noneluted material from GC	0.0	4.2	10.9	20.0	14.8

[a]Results are expressed as the percentage composition of fatty acid methyl esters determined by using Fat & Oil reference mixtures No. 3 (Applied Science Lab., State College, PA) as a standard.

chloroform/methanol (2:1, v/v) and determined gravimetrically. Liver lipid fatty acid composition was estimated by GC (11) and tocopherols were determined by HPLC (26).

RESULTS

Characteristics of Test Oils

Analytical values of the test oils are listed in Table 2, and the fatty acid composition of test oils is given in Table 3. COV, AV and the content of polar materials in each oil increased markedly during the frying period. The maximum values of COV and polar materials were obtained at 87.5 hr (heated time), while the AV and viscosity continued to increase with prolongation of the heating time. Some of the thermally oxidized materials were probably adsorbed from oil into the fried food between periods C and D. The fate of such materials should be traced by further experiments.

The fresh rapeseed oil was rich in oleic (57.2%), linoleic (20.7%) and linolenic (14.6%) acids. Erucic acid was present at a low level (2.2%). Decreases in the contents of unsaturated fatty acids were large during the frying period, especially linolenic acid. Noneluted material on GC represents polar substances in the fatty acid methyl esters; the amounts increased when the heating time was prolonged. C_{18}-Cyclic monomers were detected at low levels (fresh oil, 0%; thermally oxidized oil A, 0.04%; B, 0.13%; C, 0.22%; D, 0.18%).

The consistency of the test oils during the feeding study was confirmed by the determination of COV and POV each time a fresh batch of experimental diet was prepared; these values remained constant.

Fresh rapeseed oil contains natural tocopherols (α, 136 μg/g; γ, 368 μg/g; δ, 3.6 μg/g). Thermally oxidized oils contained no tocopherols. To determine whether added dl-α-tocopherol acetate was decomposed by contact with deteriorated oils or not, prepared diets were extracted with ether immediately and again 1 week after preparation,

TABLE 4

Results of Feeding Study[a]

Criteria	Control (n=8)	Heat-treated A (n=8)	Heat-treated B (n=5)	Heat-treated C (n=6)	Heat-treated D (n=6)	Commercial diet (n=7)
Body wt. gain (g)	201 ± 19	211 ± 12	205 ± 14	204 ± 9	218 ± 24	-
Food intake (g)	958 ± 72	1016 ± 60	974 ± 52	986 ± 35	1054 ± 82*	-
Feed efficiency[b]	0.210 ± 0.010	0.208 ± 0.010	0.210 ± 0.007	0.207 ± 0.005	0.206 ± 0.009	-
Relative liver wt. (%)	3.31 ± 0.12	3.39 ± 0.14	3.42 ± 0.11	3.74 ± 0.09**	3.47 ± 0.10*	3.08 ± 0.16
Relative kidney wt. (%)	0.60 ± 0.03	0.61 ± 0.02	0.64 ± 0.02**	0.70 ± 0.01**	0.68 ± 0.03**	0.55 ± 0.01

[a]Fresh rapeseed oil was used for the control diet. The characteristics of fresh oil and heat-treated oils (obtained from a manufacturer of fried fish paste) are given in Table 2. Rats were fed ad libitum for 13 weeks with diet which contained 15% of a test oil. Each value is the mean ± S.D. n=Number of rats examined.

[b]Feed efficiency=Body wt. gain/Food intake.

*Significantly different from control group, $p<0.05$.

**Significantly different from control group, $p<0.01$.

TABLE 5

Results of Feeding Study (Continued)

Criteria	Control (n=8)	Heat-treated A (n=8)	Heat-treated B (n=5)	Heat-treated C (n=6)	Heat-treated D (n=6)	Commercial diet (n=7)
TBA-reactive substance						
(MA, nmoles/g liver)[a]	210 ± 33	234 ± 32	277 ± 81	473 ± 43**	470 ± 58**	149 ± 19
(MA, nmoles/g liver)[b]	87 ± 8	93 ± 9	110 ± 20*	160 ± 13**	160 ± 22**	74 ± 7
(MA, nmoles/g kidney)[b]	110 ± 9	117 ± 9	109 ± 11	109 ± 5	102 ± 7	133 ± 17
(MA, nmoles/ml serum)[c]	3.7 ± 0.4	3.9 ± 0.6	3.4 ± 0.9	3.4 ± 0.3	3.2 ± 0.6	2.8 ± 0.7
GSH (μmoles/g liver)	1.06 ± 0.28	1.44 ± 0.28*	2.26 ± 0.59**	2.69 ± 0.52**	2.37 ± 0.44**	0.83 ± 0.06
GSH-px activity in liver[d]	105 ± 10	100 ± 7	84 ± 10**	88 ± 7**	80 ± 8**	116 ± 5
GSH-red activity in liver[d]	7.1 ± 2.4	6.1 ± 0.5	5.9 ± 0.9	6.4 ± 0.1	5.7 ± 0.9	-
Lipid content in liver (%)	4.8 ± 0.6	4.3 ± 0.5	4.7 ± 0.8	3.9 ± 0.3*	4.6 ± 0.5	4.6 ± 0.5
Tocopherol content						
(μg/g liver)	α,33.9 ± 2.8 γ,10.2 ± 1.9	22.1 ± 3.7**	15.9 ± 1.3**	7.7 ± 1.1**	8.9 ± 1.4**	42.5 ± 1.5
(μg/ml serum)	δ, 7.1 ± 1.0	5.8 ± 0.8	4.7 ± 0.4*	3.0 ± 0.3**	3.3 ± 0.2**	6.0 ± 0.4

[a]Measured by Ohkawa's method (21).

[b]Measured by Uchiyama's method (22).

[c]Measured by Yagi's method (18).

[d]μmoles NADPH oxidized/g liver/min.

and the extracts were saponified. Unsaponified materials were analyzed by HPLC. More than 96% of added dl-α-tocopherol acetate in all diets remained after 1 week.

Results of Feeding Study

Body weight gain, food intake, feed efficiency, relative liver weight and relative kidney weight are shown in Table 4.

Every diet was consumed in a normal manner. Food intake in the groups given heated oil was larger than that of the control group, but the body weight gain in all groups was essentially the same. No steatorrhea was observed throughout the feeding study. Significant differences were found in relative liver and kidney weight between the control group and groups given heated oil. The content of TBA-RS and other biochemical criteria are given in Table 5.

TBA-RS in liver homogenate, as determined by both Ohkawa's method (21) and Uchiyama's method (22), increased significantly in the groups given heated oil. TBA-RS in the kidney and serum were unchanged. Reduced GSH in the liver increased significantly in the groups given heated oil. GSH-px activity (including GSH-S-transferases) in the liver decreased significantly, while GR activity was unchanged.

Lipid content in the liver was unaffected except in group C. Tocopherols in the liver and serum were decreased considerably in proportion to the level of deterioration of the supplied oil. The fatty acid composition of liver lipid is given in Table 6. In the groups given heated oil, the relative amounts of palmitic (16:0), stearic (18:0), arachidonic (20:4) and docosahexaenoic (22:6) acids increased significantly, while oleic (18:1), linoleic (18:2) and linolenic (18:3) acids decreased.

DISCUSSION

There are a number of methods available for measuring lipid peroxidation in vivo and in vitro (e.g. determination of diene conjugation, TBA-RS, fluorescent products and exhalation of ethane and/or pentane in the breath). Among these methods, TBA assay has been widely used as a sensitive and simple method for animal tissues, although it is affected not only by the amount of malonaldehyde and/or lipoperoxides but also by the polyunsaturated fatty acids (PUFA) content, antioxidant level and iron catalyst in the tissue.

In many respects, lipid peroxidation and biomembranes are intimately related. Phospholipids composing the lipid bilayer of biomembranes are rich in PUFA, and oxidative attack results in the formation of PUFA radicals. These PUFA radicals absorb molecular oxygen to yield peroxy radicals and/or peroxides.

Lipid peroxides generated in biomembranes are bound to membranous protein. The combined peroxidative changes of several chemical species in fresh tissues homogenates can be measured by means of the TBA assay procedures proposed by Masugi and Nakamura (27), Ohkawa et al. (21) and Uchiyama et al. (22). Malonaldehyde precursors combined in lipid-protein complexes were measured by adding surfactant (21,27) or adjusting the pH (2.0) of the reaction mixture (22). According to

TABLE 6

Fatty Acid Composition of Liver Lipid[a]

	Control (n=3)	Heat treated A (n=3)	B (n=3)	C (n=3)	D (n=3)	Commercial diet (n=2)
14:0	0.5	0.4	0.3	0.3	0.4	0.5
16:0	17.7	18.3	17.6	18.5	20.5[b]	23.8
16:1	1.7	1.6	1.6	1.8	1.9	1.9
18:0	9.8	11.6	12.7[b]	13.5[c]	12.2[b]	10.3
18:1	34.9	32.5	31.7[c]	30.0[c]	32.8	13.5
18:2	13.2	11.7[b]	10.8[c]	9.2[c]	9.0[c]	21.1
18:3	3.0	2.3[b]	1.7[c]	1.3[c]	1.3[c]	1.2
20:3	0.6	0.6	0.7	0.6	0.6	1.1
20:4	11.1	13.0	14.8[b]	16.5[c]	13.9[b]	15.2
20:5	1.1	1.0	0.8[b]	0.8[b]	0.6[c]	0.8
22:4	1.1	1.1	1.0	1.0	0.8[b]	2.3
22:6	3.9	4.6[b]	4.9[c]	5.3[c]	4.7[b]	5.7

[a]Results are expressed as mean percentage composition of fatty acid methyl esters prepared from total liver lipids; n = number of animals examined.
[b]Significantly different from control group, $p<0.05$.
[c]Significantly different from control group, $p<0.01$.

Masugi and Nakamura (27) and Mihara et al. (28), the preexisting malonaldehyde level was rather low, and intact PUFA remained substantially unchanged while "injured" PUFA changed to TBA-RS. On the other hand, Matsushita et al. (29,30) studied the process of coloration in the TBA test of purified fatty acid monohydroperoxide. They demonstrated that metal salts, such as reduced iron, are useful for the release of TBA-RS from lipid hydroperoxide, and that oxygen is not needed for this reaction (31). They also suggested that prolonged heating in air with no addition of antioxidant could cause artificial autooxidation of coexisting native PUFA (32).

In this work, we used Ohkawa's (21) and/or Uchiyama's (22) methods. Since these methods require over 30 min for maximal color development for tissue homogenate, the possible occurrence of in vitro peroxidation could not be excluded. However, in vitro peroxidation during color development, as in the incubation of homogenate prior to TBA coloration, could be considered as the kind of reflection of peroxidative deterioration occurring in vivo.

The amounts of TBA-RS in the livers of rats of groups C and D were approximately twice that of the control. A good correlation was found between the results obtained by these two methods for fresh liver homogenate ($r=0.9770$, $p<0.001$).

Rats given the heated oil showed relative decreases of C-18 unsaturated fatty acids (18:1, 18:2, 18:3) and relative increases of arachidonic (20:4) and docosahexaenoic (22:6) acids in liver lipid. These increments in arachidonic and docosahexaenoic acid might possibly affect the results of TBA assay. However, in the group fed the commercial diet (Charles River CRF-1), in spite of the abundance of the PUFA, the TBA-RS level was very low. This result indicates indirectly that the rise of TBA-RS is not necessarily attributable to changes in the relative ratio of PUFA's in liver lipid or to artifactual formation during the reaction period.

That dietary vitamin E participates in lipid peroxidation in vivo (33) is well known. The experimental diet contained 90 IU/kg of vitamin E as dl-α-tocopherol acetate. Fresh oil contained α-tocopherol at a level of 136 μg/g, and it made up 15% of the diet, so the control group received about 120 IU/kg diet. The heated oils contained no tocopherol, so groups A-D were fed a 90 IU/kg diet. No decomposition of added dl-α-tocopherol acetate occurred in the experimental diet during the feeding test. For the normal rat or mouse, 15 IU/kg of vitamin E in the diet appears to be adequate (34). Though the vitamin E in the diet was more than sufficient in this experiment, the α-tocopherol content in the liver and serum decreased considerably as the deterioration level of the supplied oil was increased. The control group showed higher tocopherol levels in the liver and serum than the groups A-D. A graded decrease of hepatic and serum α-tocopherol levels was found in spite of the similar levels of dietary tocopherol in groups A-D, a fact of great importance. α-Tocopherol content and TBA-Rs in the liver were well correlated ($r=0.8542$, $p<0.001$.).

These observations suggest two possibilities: some oxidation products in heat-treated oil decomposed α-tocopherol in the gastrointestinal tract as the result of peroxidation in the intestinal lumen or they consumed α-tocopherol in liver biomembranes as the result of radical generation and subsequent lipid peroxidation. To clarify which actually occurs, the α-tocopherol absorption through intestinal tract with coexisting heated oil should be investigated further.

GSH is widely distributed in animal and plant cells. It plays an integral role in many biological functions, including the protection of cell membranes, the destruction of peroxides, the destruction of radiation-induced free radicals and the detoxification of xenobiotics. Wirth and Thorgeirsson (35) studied the synthesis and degradation of GSH in the rat liver by i.p. administration of diethylmaleate. They reported that liver GSH levels in normal adult rats were rapidly decreased to 15% of the control level 30 min after diethylmaleate administration and remained maximally depleted for 4 hr, after which they began to rise rapidly, returning to normal at 6 hr and to 200% normal at 24 hr. In our study, GSH levels in the livers of rats given heated oil were significantly increased, and GSH was probably consumed by a secondary oxidizing agent produced in the liver after the heated oils were ingested and an overshooting of resynthesis subsequently occurred. GSH and TBA-Rs in the liver were correlated ($r=0.6293$, $p<0.001$).

A "glutathione peroxidase system," consisting of GSH-px, GR and glucose-6-phosphate dehydrogenase, may function as a metabolic unit in the reduction of peroxides. Hepatic GSH-px (including GSH-S-transferases) activity was decreased in groups given heated oil, while GR activity was unchanged. In this study, experimental diets were supplemented with selenium (0.5 ppm). Reddy and Tappel (36) have shown that autooxidized lipid does not stimulate hepatic GSH-px when the diet is supplemented with selenium.

Since peroxide itself is normally present at low levels in heated fats, and its lymphatic absorption is very low, nonvolatile oxidized products accumulated during prolonged heating are important in assessing the biological effects of thermally oxidized oil. Combe et al. (37) reported that nonvolatile oxidized products, such as polymeric acids, oxidized monomeric acids and cyclic monomeric acids can be recovered from the lymphatic lipids. Accord-

ing to Artman and Smith (38), 136 components (0.42% of the fat) were separated from potato chips fried in cottonseed oil (182 C, 48 hr) and 51 components were characterized. Although describing the biological effects of heated fat with a single index might be difficult, the existing data from many feeding studies show that polar components, such as ox.FA, column chromatographically separated polar fraction (CC-PF), GD and cyclic monomers are the major factor responsible for the toxic effects.

On the basis of the linolenic acid content (14%) and heating conditions (180 C, in air), formation of C_{18}-cyclic monomers in this study was expected to be rather low, and was confirmed to be less than 0.22% by GC analysis. The German Society for Fat Research has recommended a level of 1.0% or more of ox.FA to aid sensory evaluation (39) in the quality assessment of used frying fats. Since the method of deterioration of ox.FA is time-consuming, a column chromatographic analysis was developed by Gupta (40) for the determination of polar components. Billek et al. (39) proposed that 1% of ox.FA corresponds to 27% (by weight) of polar components. On the other hand, Billek et al. (41) conducted feeding experiments with heated sunflower oil that had been used for the industrial production of fish fingers. The polar fraction was separated by means of column chromatography on silica gel, and then fed to rats at a concentration of 20% in the diet over a period of 18 months. Although the polar fraction caused a lower weight increase of the test animals, it showed no serious effects according to many biological, clinical and histological investigations. They calculated "a tentative ADI" of the polar fraction as 10 g/70 kg/day. Ohfuji et al. (42) proposed 1% GD formation in heated fats as an index of deterioration.

Using ox.FA, CC-PF and GD as indices of the quality of heated oils is reasonable, since good correlations ($p<0.001$) exist between these indices and TBA-RS (ox.FA, $r=0.9191$; CC-PF, $r=0.9056$; GD, $r=0.9023$). Though viscosity ($r=0.9205$) and IV ($r=-0.8796$) also showed good correlations with TBA-RS, these indices differ greatly in different sort of fats, and may not be suitable for adoption as general indices.

Since determining ox.FA, CC-PF and GD were very time-consuming, many simple and convenient methods have been developed. Urakami et al. (14) noticed that most of the components detected by Artman and Smith (38), and regarded as toxic, have a strong absorption at 233 nm, and they proposed a TLC-UV assay for assessment of the P/Ap ratio. P/Ap showed a good correlation with TBA-RS ($r=0.8296$). Relative interface tension (15) also showed a good correlation with TBA-RS ($r=-0.8123$).

The carbonyl value of heated oils is an accepted index of the degree of degradation from oxygen attack, and the high carbonyl values of the oxidized oils indicate that attack by molecular oxygen during heating produced considerable amounts of carbonyl materials. Uchiyama and Sato (43) pointed out that carbonyl compounds formed by the deterioration of oils, such as 12-keto oleic acid, have a prooxidant action on unsaturated fatty acids in vitro and, when ingested by animals, they might participate in lipid peroxidation in vivo. Budowski et al. (44) found that thermally oxidized safflower oil is very effective in producing nutritional encephalopathy when fed to young chicks in a diet deficient in vitamin E. These results suggest that unsaturated keto compounds formed in heated oil may cause lipid peroxidation in vivo. Carbonyl value also showed a good correlation with TBA-RS in the liver ($r=0.8647$).

According to Hemans et al. (45) and Iwaoka and Perkins (46), the results of this type of experiment depend on dietary protein level with the adverse effects of "used" fats progressively ameliorated by increasing levels of protein. Since the dietary protein level in this study was rather high (22.6%), food intake and body weight gain were found to be substantially the same in all groups. Even though the feeding study was carried out under very mild conditions, TBA-RS, GSH, vitamin E and the fatty acid profile clearly changed in proportion to the degradation indices of the supplied oils. These results suggest that some substances present in thermally oxidized oils act to promote peroxidative deterioration in the liver. We considered that the investigation of such criteria is a valuable experimental model for quantitative assessment of possible chronic effects of thermally oxidized oils. Further work is necessary to determine whether or not these results are universally applicable to used oils. Confirmation of the present results is also desirable by the use of other techniques, such as the measurement of diene conjugation, lipofoscin and/or exhalation of short-chain hydrocarbon gases in the breath.

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