Quality and Consumption of Oxidized Lipids from Deep-Frying Fats and Oils in New Zealand

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ABSTRACT: Samples of beef dripping and plant oil-based deep-frying fat were obtained from fast-food premises in Christchurch, New Zealand, as well as samples of deep-fried battered fish and potato chips. The fat in these samples was analyzed for level of oxidation by measuring acid value, peroxide value, polar compounds, thiobarbituric acid-reactive substances (TBARS), and conjugated dienes. The acid and peroxide value results were contradictory when compared with limits set in the New Zealand Food Regulations 1984, but the levels of polar compounds were generally below 25%, indicating that the fats were well maintained. The TBARS and conjugated diene levels were compared with those for oxidized corn oil used in a feeding trial and indicated a similar oxidation level, although the amount of fat consumed in the feeding trial would be 30–50% higher. These results show that well-maintained deep-frying fat has oxidation levels sufficient to cause elevation of plasma lipid oxidation levels as observed in a human feeding trial.

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KEY WORDS: Animal fat, beef dripping, consumption, deep frying, lipid, oxidation, plant oil.

Oxidized lipoproteins are believed to play an important role in the development of atherosclerosis (1,2). It also has been suggested that dietary oxidized lipids may contribute to this process (3,4). There is evidence from animal studies that ingestion of oxidized oils and lipid hydroperoxides is more atherogenic than ingestion of unheated oils (5). Elevation of the level of oxidized lipids in plasma for several hours after ingestion of oxidized lipids has been shown in a number of studies in animals (6,7) and humans (8,9). A more recent report has shown acceleration of the development of fatty streaks in rabbits fed a diet containing 5% oxidized corn oil, compared with rabbits fed a diet with 5% unoxidized corn oil (10). The transfer of oxidized fatty acids from chylomicrons into very low-density lipoprotein in the rat has also recently been demonstrated (11).

Although the mechanism for absorption of dietary oxidized lipids remains to be elucidated, it is apparent that such food components can affect physiological oxidation levels. In the present study, the quality of deep-frying fats and oils in use in fast-food retail outlets in Christchurch, New Zealand, was investigated by using a variety of common measurements for oxidation level. The purpose was to compare levels of oxidized lipids in actual fast foods with the levels used in a human feeding trial (8). In addition, the level of oxidation in animal fats was compared with the level in samples of plant-based oils.

EXPERIMENTAL PROCEDURES

Materials. Butylated hydroxytoluene (BHT) and 1,1,3,3-tetraethoxypropane were obtained from Sigma (St. Louis, MO) The conjugated diene standard, 9-hydroxy-10,12-octadecadienoic acid, was obtained from Cayman Chemical (Ann Arbor, MI). Solvents were of analytical grade and obtained from either BDH Laboratory Supplies (Poole, England) or Mallinckrodt Specialty Chemicals Co. (Paris, KY). Samples were obtained from fast-food retail outlets in Christchurch during November 1995. Samples of frying media were obtained directly from the frying vat in use; approximately 100 g was poured into a 100-mL Schott bottle (Duran, Mainz, Germany) that contained 100 mg BHT (final antioxidant concentration 0.1%). Samples of deep-fried potato chips (french fries) or battered fish were stored frozen under nitrogen until extraction (within 2–3 d). The animal fat samples were all beef dripping. The plant oils used included four of soybean oil, one of palm oil, and several commercial brands of unknown origin. These commercial brands were all clearly stated to be entirely vegetable or plant oil-based.

Extraction. The food samples were extracted with a mixture of chloroform and methanol according to a published procedure (12). Antioxidant (BHT; 75 ppm) was added to the solvent so that the final concentration in the extracted fat was 0.1%.

Fat quality analyses. American Oil Chemists' Society (AOCS) methods (13) were used for determining acid value (Method Cd 3d-63), peroxide value (Method Cd 8-53), and polar compounds (Method Cd 20-91). A solution of hydrogen peroxide was standardized against thiosulfate (14) and used to demonstrate that the peroxide test was giving accurate results. Chromatographic resolution of components in the polar compounds tests was checked by thin-layer chromatography.

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Thiobarbituric acid-reactive substances (TBARS). A TBARS stock standard (1,1,3,3-tetraethoxypropane; 1 mM) was prepared in ethanol/water (40:60), and further dilutions were made (usually three or four standards in the range 0.5–50 μM) each week. Samples were prepared by dissolving approximately 100 mg of fat or oil in distilled *n*-butanol (20 or 25 mL) as in AOCS Method Cd 19-90 (13).

Analysis was performed according to the method of Morel *et al.* (15). A mixture of sample or standard (0.1 mL), aqueous trichloroacetic acid (20%, 1 mL) and aqueous thiobarbituric acid (TBA; 1%, 1.0 mL) was heated at 95˚C for 45 min and then cooled. Quantitation was performed by a high-pressure liquid chromatography (HPLC) procedure (16) (Waters 600 multisolvent delivery system, Milford, MA; Hitachi F1000 fluorescence spectrophotometer, Tokyo, Japan; Spectraphysics SP4270 integrator, San Jose, CA). A C-18 column (25 cm × 4.6 mm i.d., Brownlee; Applied Biosystems Inc., Foster City, CA) was eluted with phosphate buffer/methanol (58:42, 1 mL/min, 50 mM phosphate buffer at pH 6.0). Fluorescence detection of the TBA red-colored adduct (which eluted at about 5–6 min) was performed with excitation at 515 nm and emission at 553 nm (17).

Samples were neutralized to pH 6–7 with sodium hydroxide (1 M) immediately prior to injection (injection volume 25 μL). Sample results were corrected for a butanol blank and then read against a standard curve, generated from the standard results run each day (corrected for an ethanol/water blank).

Conjugated dienes (CD) determination (18). Dilutions of 9-hydroxy-10,12-octadecadienoic acid (100 μg in 0.1 mL ethanol) in Analar 96% ethanol/*n*-butanol (96:4) were used as standards. Normal and second-derivative ultraviolet spectra were run from 190–300 nm (Shimadzu UV240 UV-visible recording spectrophotometer, Kyoto, Japan; Shimadzu PR-1 graphic printer with OPI-1 option program/interface). Standard concentrations were calculated from the normal spectrum (maximum at 233 nm; extinction coefficient 2.3×10^4). The calibration line was created from the summed intensities of peaks in the second-derivative spectra taken from minima at 233 and 242 nm to the next maxima (at longer wavelength). Samples (in the same solvent) were analyzed in the same way.

RESULTS AND DISCUSSION

Means, standard deviations, and range values of results from the various analyses of fats and oils are given in Table 1.

Fat quality analyses. The New Zealand Food Regulations 1984 (19) requires that nonvirgin fats and oils have an acid value of not more than 0.6 mg KOH/g (or 2 mg KOH/g if beef dripping) and that the peroxide value not exceed 10 milliequivalents oxygen/kg [Regulations 87 (5) (a) and (b), and 89]. Only 5 of the 18 fat samples (both animal- and plant-based) complied with the acid value limits, but all complied with the peroxide regulation.

Although there is no New Zealand regulation based on the level of polar compounds, this is a common measure of fat deterioration in Europe (20). Generally, a level of 25–27% polar compounds is regarded as an upper limit. While the results for polar compounds are generally higher for plant-based oils than for animal fats, they are still well below the suggested limit of 25–27% (in fact, only two samples had polar compounds

TABLE 1
Results from Analysis of Food and Fat Samples for Oxidation Level

Sample type (number of samples)	Fat content (%)	Acid value (mg KOH/g)	Peroxide value (millieq. oxygen/kg)	Polar compounds (%)	TBARS[a] (μmoles/g)	Conjugated dienes (nmoles/mg)
Fish (animal fat) (4)						
Mean	19.1	2.8	3.4	11.8	0.3	26
Standard deviation	4.1	0.7	1.7	1.5	0.2	3
Range	13.0–21.9	2.1–3.6	2.2–5.9	10.4–13.8	0.08–0.48	23–30
Fish (plant oils) (3)						
Mean	14.1	2.1	1	20.1	0.15	22
Standard Deviation	2.8	0.5	0.2	6	0.07	9
Range	12.1–16.0	1.6–2.6	0.8–1.1	16.6–27.0	0.11–0.23	13–31
Chips (animal fat) (5)						
Mean	12.7	2.6	2.9	13.4	0.04	20
Standard deviation	2.4	1.7	1.4	4.5	0.01	3
Range	10.1–15.3	0.6–5.2	1.1–4.5	7.6–19.1	0.03–0.05	14–22
Chips (plant oils) (3)						
Mean	12.5	4.4	1.3	16	0.12	24
Standard deviation	2.2	5.9	0.5	6.1	0.08	11
Range	11.1–15.0	0.6–11.2	0.9–1.8	12.5–23.0	0.07–0.22	13–24
Animal Fats (9)						
Mean		2.9	2.9	16	0.05	26
Standard deviation		1.7	1.7	4	0.02	5
Range		0.4–5.9	1.3–5.7	10.0–24.0	0.03–0.09	20–34
Plant Oils (9)						
Mean		3.3	1.7	20	0.11	25
Standard deviation		3.8	0.7	6	0.1	10
Range		0.3–12.6	1.1–3.2	12.0–31.0	0.03–0.31	12–41

[a]TBARS, thiobarbituric acid-reactive substances.

greater than 25%). These results indicate that the fats in use were generally of good quality and that the maintenance procedures (partial fat replacement, filtering) used by the frying operators were effective in maintaining quality.

Comparison of animal fats with plant-based oils. Plant-based oils, with their higher unsaturated content, are more susceptible to autoxidation than animal-derived fats. However, in terms of acid and peroxide values and levels of polar compounds, this study found relatively little difference between the animal fats and plant-based oils that were taken directly from the frying vats. Statistical analysis of these sets of results showed that none were significantly different at the 5% level (two-tailed Students' *t*-test for either same or different variances, depending on F-test analysis). In addition, the similarity of results for the animal fats, compared with plant oils, indicates that the choice of foods fried in plant-based oils would have relatively little effect on intake of oxidized lipids. This suggests that such a choice could be justified on other nutritional considerations, such as reduced saturated fat intake.

TBARS. There are two reports in which TBARS results were determined by methods similar (but not identical) to that used in this study. A mixture of 90% tallow/10% cottonseed oil was used for deep-frying potato chips at approximately 170˚C (21). Details of the method are not given, but TBARS expressed as parts per million rose from 0.27 to a maximum of 0.73 after 80 h use. Converting these mg/kg results to μmoles of malondialdehyde/g gives 0.004–0.010 μmole/g. The results from our study are markedly higher than this. The other report gave the TBARS values for a series of unused plant-based oils (22). Values ranged from 0.2–0.6 μmoles/g, which is generally higher than our results. However, the TBARS measurements were made by using absorbance without HPLC separation, and this may account for the differences in results.

For all analyses, the results for fat or oil extracted from potato chips are similar to those for fat taken directly from the vats. This suggests that fat absorbed by the potato chips during deep-frying does not markedly change in quality. The TBARS values for the fat extracted from fish deep-fried in animal fats are considerably higher than the samples taken directly from the vats, and slightly elevated in the fat extracted from fish that was deep-fried in plant-based oils. It is likely that the highly unsaturated native fish oils contribute to the TBARS level, although this is not reflected in the CD values.

CD. The CD results for fats and oils derived from fast foods in Christchurch are surprisingly consistent at between 20–30 nmol conjugated diene/mg. The CD measurement in the animal fats will be increased by natural levels of preformed conjugated linoleic acid (23). This may account for the similarity in the level of CD in the beef dripping samples with the plant oils, despite their lower TBARS values. This measurement of oxidation is less useful for these animal fat samples.

Comparison with lipids used in human feeding trial. As shown by the comparisons with literature results, the TBARS measurement is subject to many variables, which make comparisons between reports difficult (24). In this project, the TBARS analytical method (15) and the CD measurement (18) were chosen to allow comparison with the levels of oxidized lipids measured in the corn oil used in a human feeding trial (8), which demonstrated elevated levels of oxidized lipids in chylomicrons after subjects consumed either "medium" or "highly oxidized" corn oil. The TBARS and CD values for "medium" and "highly oxidized" corn oil are shown in Table 2. The mean TBARS value for plant-based oils in this study was equivalent to "highly oxidized" corn oil. Comparison of the corn oil results with the TBARS values obtained from animal fats in this study may not be appropriate. It is unclear whether the lower TBARS values in the animal fats reflect a lower level of oxidation (similar to "medium" oxidized corn oil in the feeding trials) or simply the differing fatty acid composition of the animal fat compared with plant-based oils.

By using CD values for comparison, the plant oils in this study would rank as similar to the "medium" oxidized category. However, when the TBARS values are considered, the CD levels for the plant-based oils could have been expected to be higher.

Consumption of oxidized lipids. Consumption data for fast foods in New Zealand from the 1990/91 Life in New Zealand Survey (24-h dietary recall) indicated that, for those respondents who consumed french fries in the preceding 24-h period, the mean weight consumed was 175 g (25). Using the mean fat content of chips from our study, this indicates a fat consumption from potato chips, fried in animal fat and plant oils, of 22.2 g and 21.9 g, respectively. Data from the same survey for battered fish gave the mean weight consumed as 136 g (26). Using the mean fat content of fish from our study, this indicates a fat consumption from deep-fried fish, fried in animal fat and plant oils, of 26.0 g and 19.2 g, respectively. This gives an estimate of total fat consumed from an average meal of "fish and chips," when fried in animal fat, of 48.2 g, and

TABLE 2
Comparison of Results for Plant-Based Oils and Corn Oil Used in Feeding Trial (Ref. 8)[a]

	TBARS (μmoles/g)	Conjugated dienes (nmoles/mg)	Consumption (g/kg)	Conjugated dienes in chylomicrons (nmoles/μmol triglycerides)
Plant-based oils (n = 9)	0.11	25	0.5–0.6	
Feeding trial corn oil				
Unoxidized	n.d.	6.5–10	1	10
Medium oxidized	0.041	30–50	1	22
Highly oxidized	0.103	80–120	1	46

[a]See Table 1 for abbreviations.

when fried in plant oils, of 41.1 g.

In the human feeding trial (8), subjects consumed corn oil equivalent to 1 g/kg body weight. On the basis of the average weight of males and females in New Zealand (27), this would equate to a fat intake of 78 and 65 g, respectively. The amounts of fat estimated for an average New Zealand "fish and chips" meal would represent an intake of 0.5–0.6 g/kg body weight. Depending on whether TBARS values or CD are used as an indicator of the degree of oxidation, the plant oils in this study could be considered as equivalent to either the "medium" or "highly oxidized" corn oil. Thus, the intake and oxidation level of actual fat from deep-fried fish and potato chip meals would be expected to give a lesser increase in chylomicron CD levels than those observed in the trial. The values of oxidation measures, consumption, and increases in oxidation levels in plasma are shown in Table 2. However, from the range of results in Table 1, it would not be unreasonable for situations to occur where above-average consumption, fat content, or oxidation level would combine to produce intakes comparable to those in the feeding trial.

The significance of the observed increases in plasma oxidation levels to the atherosclerotic process has yet to be determined. However, the results of this study demonstrate that dietary oxidized lipid intakes, which increase oxidized lipid levels in plasma, can be derived from foods deep-fried in fats or oils that are being well maintained as judged by the measurement of polar compounds.

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