Digestibility of Fatty Acid Monomers, Dimers and Polymers in the Rat

G. Márquez-Ruiz, M.C. Pérez-Camino and **M.C. Dobarganes***
Instituto de la Grasa y sus Derivados, C.S.I.C., 41012-Sevilla, Spain

This study was designed to determine digestibilities of fatty acid monomers, dimers and polymers as components of diets containing thermally oxidized oils. Male Wistar rats were fed semipurified diets supplemented with unheated, heated and a 1:1 mixture of unheated/heated olive oils at 6, 12 and 20% w/w of diet. In a 14-d experimental period, fecal lipids were extracted and analyzed by a combination of adsorption and high-performance size-exclusion chromatographies. Thus, it was possible to separate and quantitate five groups of fatty acids—nonpolar monomers, oxidized monomers, nonpolar dimers, oxidized dimers and polymers. Nonpolar fatty acid monomers showed high digestibilities, although significantly influenced by the alteration level of the dietary oil. The apparent absorption of oxidized fatty acid monomers averaged 76.6%. Among polymeric fatty acids, the lowest digestibilities were found for nonpolar dimers (10.9% on average), whereas oxidized dimers and polymers possessed higher apparent absorbability than expected, ranging from 22.7% to 49.6%. Chemical modifications prior to absorption, leading to less complex products, may have contributed to enhanced digestibility of polymers.

KEY WORDS: Digestibility, fatty acid polymers, fecal lipids, high-performance size-exclusion chromatography, nonpolar fatty acids, nonpolar fatty acid dimers, oxidized fatty acid dimers, oxidized fatty acid monomers, rat, thermoxidized oil.

The biological effects that consumption of heated and/or oxidized fats may exert on human health have stimulated extensive research in the past years (1–6). However, there is considerable controversy regarding the nutritional significance of the degradation compounds formed because experimental conditions vary widely—different oils and heating conditions (time, temperature, aeration, time periods), with or without the presence of food. Animal studies associated with the feeding of frying oils, which are probably the main dietary source of thermally oxidized fats, have not generally revealed detrimental effects (7–10), with the exception of those studies conducted on concentrated fractions of altered compounds (11–14).

Overall, reports on digestibility have indicated significantly lower values for thermally oxidized oils compared to fresh oils, and this fact generally has been attributed to the presence of the nondistillable urea-nonadductable fraction (15,16), which essentially contains polymeric products. In addition, experiments carried out on purified compounds derived from thermally oxidized oils have reported digestibilities ranging from 30–70% for dimers (17–19), although such high values have been questioned (20). No data have been found for cyclic monomer digestibility, presumably because these compounds are readily absorbed and, hence, difficult to detect in feces (3,21).

Nevertheless, it must be realized that complex mixtures of lipid degradation products occur in our daily diet, and further investigation is needed in this regard. Unfortunately, because of the complex mixture of such alteration products and the lack of specificity of the analytical methods used, the determination of specific compounds has been difficult to date.

In this context, the aim of the present study was to determine the digestibilities of fatty acid monomers, nonpolar and oxidized fatty acid dimers and polymers in rats fed thermally oxidized olive oils. For this purpose, we used an analytical procedure based on adsorption and size-exclusion chromatographies, which allowed separation and quantitation of five groups of compounds in dietary oils and fecal lipids (22).

MATERIALS AND METHODS

Animals and diets. Sixty male Wistar rats, weighing about 120 g, were randomly assigned to 10 groups and fed diets containing pure commercial olive oil, oil heated at 180°C for 150 h and 1:1 unheated/heated mixture, each provided at three levels of 6, 12 and 20% w/w on diet. One group of animals received a fat-free diet for the determination of endogenous fat. The composition of the basal fat-free semipurified diet was 10% water, 19.8% protein, 57.6% carbohydrates (nitrogen free extract), 5.4% cellulose, 6.2% mineral mixture and 1.0% vitamin mixture (Panlab, Barcelona, Spain). The chemical analysis of the olive oils added to the basal diet is summarized in Table 1.

TABLE 1

Quantitative Determination of Nonpolar and Polar Fatty Acids[a] in Dietary Oils (wt% on fat)

	Nonpolar fraction		Polar fraction		
Olive oil	Nonaltered fatty acids	Nonpolar dimers	Oxidized monomers	Oxidized dimers	Polymers
Unheated	98.2 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.3 ± 0.1	n.d.[b]
Unheated/heated (1:1)	81.5 ± 0.4	1.9 ± 0.2	4.4 ± 0.2	4.2 ± 0.1	7.0 ± 0.2
Heated	65.3 ± 0.4	3.8 ± 0.2	8.6 ± 0.2	8.1 ± 0.1	13.5 ± 0.2

[a]Means ± SEM of four determinations. [b]Non-detectable.

*To whom correspondence should be addressed at Instituto de la Grasa y sus Derivados, C.S.I.C., Avda. Padre García Tejero, 4, 41012 Sevilla, Spain.

The animals were placed in individual metabolic cages to determine food intake and to collect feces. Both diets and water were provided *ad libitum* and the weight of rats recorded periodically. The control group was given a fat-free diet for two periods of 15 days in order to determine endogenous fecal fat excretion. Upon a 7-d orientation period, the feces were collected during the following 14 d and stored at −25°C until analyzed.

Extraction of fecal lipids. The combined feces of the experimental period were dried under vacuum to constant weight, pulverized and extracted in a Soxhlet extractor with diethyl ether for 8 h (19). The residue was dried, mixed with 3N HCl and re-extracted with diethyl ether to obtain the total lipids excreted. Fecal lipids thus obtained were dried under vacuum at 60°C to constant weight.

Analytical procedure. Both dietary oils and fecal lipids were analyzed according to an analytical procedure, which includes the following stages: (i) Separation of unsaponifiable matter (23)—lipid samples were saponified, and unsaponifiable matter was removed. After acidulation with 1N HCl, fatty acids were extracted from the aqueous solution by repeated diethyl ether extractions. Following evaporation of the diethyl ether, the sample was dried under nitrogen to constant weight. (ii) Preparation of methyl ester derivatives according to Metcalfe *et al.* (24). (iii) Separation of methyl esters by means of silica column chromatography (22). Hexane/diethyl ether (88:12) was used to elute the nonpolar fraction while diethyl ether was used to obtain the polar fraction, followed by a final elution with methanol to improve recovery of the sample. Separation of nonpolar and polar fractions was performed by high-performance size-exclusion chromatography (HPSEC). The samples were analyzed in a Konik 500 A chromatograph (Konik, Barcelona, Spain) with a 10-μL sample loop. A Hewlett-Packard 1037 A refractive index detector (Hewlett-Packard, Pittsburgh, PA) and two 100 Å and 500 Å Ultrastyragel columns (Water Associates, Milford, MA), connected in series, operated at 35°C. The columns were 25 cm × 0.77 cm i.d., packed with porous, highly cross-linked styrenedivinylbenzene copolymer (10 μm). High-performance liquid chromatography (HPLC)-grade tetrahydrofuran served as the mobile phase with a flow of 0.5 mL/min, and the sample concentration was between 15 and 20 mg/mL in tetrahydrofuran.

Determination of digestibility coefficients. True digestibility (TD) was calculated as follows:

$$TD = [(\text{lipid ingested} - \text{lipid excreted corrected for endogenous fecal lipid content})/\text{lipid ingested}] \times 100$$

Corrections for endogenous contribution to fecal lipids were made by subtracting the fecal fat recovered in the group fed the fat-free diet at similar time periods from those for dietary treatments.

Statistical methods. Data are expressed as the mean and standard error of the mean. Differences between dietary treatments were assessed by Student's *t*-test, and P values < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Figure 1 shows HPSEC chromatograms of nonpolar and polar fractions of representative fecal lipids from rats fed fat-free diet and diets supplemented with 20% of unheated, 1:1 unheated/heated mixture and heated oil, respectively. The possibilities of the combined chromatographic analysis used in this study have been published recently (22). It permits the determination of five groups of compounds differing in polarity or molecular weight. Fatty acid monomers are separated into nonpolar (NP FA) and polar monomers, originating *via* oxidation (ox FA) in the first and second fractions, respectively. Otherwise, both fatty acid monomer peaks would overlap due to similar molecular weight. Likewise, nonpolar dimers (NP FAD), representative of thermal alteration as there is no oxygen involved in their formation, are quantitated in the first fraction, independently of oxidative dimers (ox FAD), which are determined in the polar fraction. Finally, polymeric compounds (FAP) are included and analyzed in the latter fraction. As can be observed, endogenous lipids are comprised of compounds with molecular weight lower than that of fatty acid dimers. Hence, interference due to endogenous contribution only affects fatty acid monomer determinations. Although endogenous fatty acids are predominantly nonpolar, polar fatty acids make up approximately 8–9% of fecal lipids, and they are largely due to colonic bacterial action and biliary sources (25–27). HPSEC chromatograms show notable differences in compound distribution when fat-free and oil-supplemented dietary groups are compared. As for the unheated oil diet, the presence of nonpolar and oxidized dimers in the first and second fractions of fecal lipids, respectively, indicates poor digestibility for both types of dimers, given the small amounts of these compounds ingested in such a diet. On the other hand, proportions of dimers and polymers substantially increase as the alteration level of the dietary oil increases.

The quantitative data of fecal lipid compound distribution is presented in Table 2, expressed as total levels excreted and as percentages of fecal lipids. Total fecal lipids were significantly higher when the alteration degree of the dietary oil was greater. Clearly, the significant differences found in lipid distribution among groups are related to dietary oil compositions. Thus, excreted levels of altered compounds—dimers plus polymers—were two-fold higher for the heated-oil diet compared to the unheated/heated oil diet, which is consistent with the ingested amounts.

Results for the digestibility measurements of nonpolar and oxidized fatty acid monomers are presented in Table 3. Digestibility values were corrected for interference due to fatty acids from endogenous sources. As expected, digestibilities of nonpolar fatty acids, *i.e.,* nonaltered fatty acids, were generally very high. Nevertheless, we found that digestibility decreased significantly as the extent of alteration of the dietary oil was greater, and independently of the dietary oil content. These results may be attributed to impaired lipolysis of altered triacylglycerols, which include, in part, nonaltered fatty acyls (28). On the other hand, the amounts of oxidized fatty acids excreted from rats fed unheated oil were undetectable, undoubtedly due to their low contribution to the diet (Table 1) along with their high digestibility. Interestingly, digestibilities of oxidized fatty acids were high for all the other dietary treatments. There were no significant differences due to oil level, whereas digestibility was lower for groups fed heated oil as compared to those fed unheated/heated oil diet. Oxidized fatty acid monomers include mainly

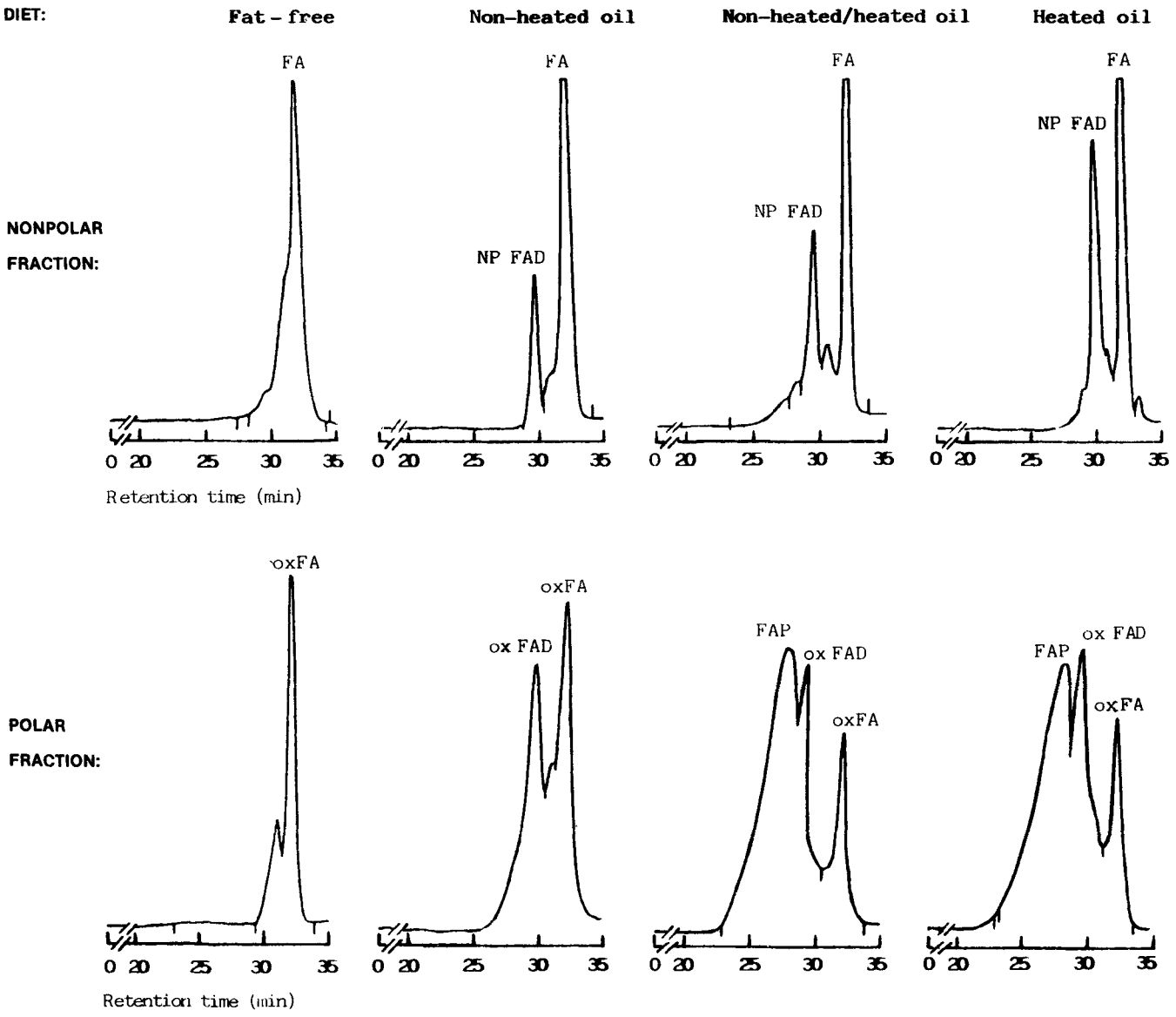

FIG. 1. Representative HPSEC chromatograms of nonpolar and polar fractions of fecal lipids from rats on fat-free diet and diets containing unheated, unheated/heated (1:1) and heated oils (20% on diet). Abbreviations: FA, nonpolar fatty acids; oxFA, oxidized fatty acids; NP FAD, nonpolar fatty acid dimers; ox FAD, oxidized fatty acid dimers; and FAP, fatty acid polymers.

compounds originating *via* oxidation at low temperature—hydroperoxy, keto and hydroxy acids, as well as polyoxygenated monomeric compounds (29). There is increasing evidence that oxidized lipids may be detrimental to health. Fatty acid hydroperoxides have been shown to accelerate all three phases of atherosclerosis—endothelial injury, accumulation of plaque and thrombosis (30–35). In addition, it has been reported that secondary products of fatty acid oxidation are absorbed into the circulation and incorporated into the liver of rats, with deleterious effects (36–42). Recent investigations also have demonstrated a role of oxidized fatty acids on the promotion of intestinal tumors based on the fact that hydroperoxy, hydroxy and enone-containing fatty acids induce ornithine decarboxylase activity and stimulate DNA synthesis in colonic mucosa *in vivo* (43–47).

Table 4 shows digestibility coefficients of fatty acid nonpolar dimers, oxidized dimers and polymers. Regarding both types of dimers, no consistent differences were found due to either dietary oil level or alteration degree. Digestibility of polymers was not significantly different among groups. In general, digestibilities of nonpolar dimers were quite low, and these results seem to indicate that such compounds, originating under high temperatures, are not easily absorbed, possibly due to their high molecular weight. In contrast, oxidized dimers showed comparatively high digestibility coefficients, ranging from 22.7% to 49.6%. Surprisingly, polymer digestibilities were similar to those obtained for oxidized dimers, despite the differences in molecular weight and structural complexity.

Overall, the results presented in Table 3 give evidence of the complexity of the absorption process. Although the

TABLE 2

Distribution of Lipid Components in Fecal Fats from Rats Fed 20% Oil on Diet During 14 Days (wt% on ingested fat)

Diet	Total fecal lipids[a]	Lipid components[a]					
		Unsaponifiable matter	Nonaltered fatty acids	Nonpolar dimers	Oxidized monomers	Oxidized dimers	Polymers
Unheated oil	3.64 ± 0.40[a] (100.0)	1.07 ± 0.07[a] (29.4)	1.42 ± 0.12[a] (39.0)	0.48 ± 0.03[a] (13.2)	0.37 ± 0.03[a] (10.2)	0.30 ± 0.03[a] (8.2)	—
Unheated/heated (1:1) oil	16.91 ± 0.41[b] (100.0)	1.25 ± 0.06[a] (7.4)	4.07 ± 0.34[b] (24.1)	1.63 ± 0.11[b] (9.6)	0.91 ± 0.06[b] (5.4)	3.02 ± 0.23[b] (17.9)	6.03 ± 0.45[a] (35.7)
Heated oil	29.87 ± 1.84[c] (100.0)	1.29 ± 0.08[a] (4.3)	6.59 ± 0.41[c] (22.1)	3.52 ± 0.16[c] (11.8)	2.30 ± 0.18[c] (7.7)	6.34 ± 0.41[c] (21.2)	9.83 ± 0.66[b] (32.9)
Fat-free[b]	0.39 ± 0.02[d] (100.0)	0.25 ± 0.02[b] (64.1)	0.11 ± 0.01[d] (28.2)	—	0.03[c,d] (7.7)	—	—

[a]Means ± SEM of six rats. Values in columns without common superscript letters are significantly different, $P < 0.05$. Values between brackets are expressed as wt% on fecal lipids.

[b]Fat recovered during 14 days.

[c]Calculated by difference.

TABLE 3

True Digestibility of Nonpolar and Oxidized Fatty Acid Monomers[a] in Rats Fed Unheated, Unheated/Heated (1:1) and Heated Oils at 6, 12, and 20% w/w on Diet

Dietary oil		True digestibility (%)	
Alteration	Oil on diet (%)	Nonpolar fatty acids	Oxidized fatty acids
Unheated	6	99.3 ± 0.2[a]	—
	12	98.6 ± 0.3[a]	—
	20	98.8 ± 0.3[a]	—
Unheated/heated (1:1)	6	96.3 ± 0.7[b]	79.7 ± 1.4[a,b]
	12	96.0 ± 0.5[b]	80.7 ± 1.5[a,b]
	20	95.2 ± 0.7[b]	81.8 ± 0.9[a]
Heated	6	89.6 ± 0.5[c]	74.5 ± 2.5[b,c]
	12	89.4 ± 0.7[c]	68.9 ± 1.9[c]
	20	90.2 ± 0.8[c]	74.3 ± 1.3[c]

[a]Means ± SEM of six rats. Values in columns without common superscript letters are significantly different, $P < 0.05$.

TABLE 4

True Digestibility of Fatty Acid Nonpolar Dimers, Oxidized Dimers and Polymers[a] in Rats Fed Unheated/Heated (1:1) and Heated Oil Diets at 6, 12 and 20% Levels (w/w on diet)

Dietary oil		True digestibility (%)		
Alteration	Oil on diet (%)	Nonpolar	Oxidized	Polymers
Unheated/heated (1:1)	6	15.5 ± 2.6[a]	38.3 ± 4.0[a,b]	33.5 ± 3.7[a]
	12	11.8 ± 2.2[a,b]	49.6 ± 5.4[a]	24.2 ± 6.6[a]
	20	12.8 ± 1.8[a,b]	34.6 ± 4.7[a,b,c]	23.6 ± 4.9[a]
Heated	6	16.3 ± 2.8[a]	27.6 ± 2.9[b,c]	36.9 ± 3.7[a]
	12	1.8 ± 1.1[c]	33.3 ± 2.3[b]	31.3 ± 2.0[a]
	20	7.4 ± 1.6[b]	22.7 ± 2.9[c]	28.9 ± 3.7[a]

[a]Means ± SEM of six rats. Values in columns without common superscript letters are significantly different, $P < 0.05$.

uptake of micellar lipid by the intestine is considered to be energy-dependent, little is known of the precise mechanisms by which lipolytic products pass from the mixed micelles into the mucosal cells (48,49).

Briefly, absorption depends on lipolytic enzyme activities in the first stages (50). Then, the entry of lipid products into the micellar phase is determined by their solubility in the bile salt solution (51), which is influenced to a great extent by the polarity of the molecule. Finally, the uptake from the lumen is substantially limited by molecular weight. Among fatty acid monomers, the reduction of nonpolar fatty acid digestibilities for heated oils may be due partly to impaired hydrolysis of altered triacylglycerols. Regarding fatty acid dimers, differences found in digestibility of nonpolar and oxidized types might be explained in terms of polarity. On the other hand, in view of the high values obtained for polymers, we suggest that chemical modifications may occur prior to absorption, possibly during digestion. This suggestion is substantiated by *in vitro* studies which show polymer breakages through ether and peroxide linkages when using strongly acidic treatments (52–54). Thus, released products from polymers of lower molecular weight could be more readily absorbed.

In summary, from the results of the present study, nonpolar fatty acid dimers are poorly absorbed while oxidized dimers and polymers show unexpectedly enhanced absorption. Finally, our observations support the view that more investigation is needed into the nutritional implications of oxidized fatty acid monomers in light of their high digestibility.

ACKNOWLEDGMENTS

This work was supported by CICYT (project ALI 88-0208). The authors thank M. Giménez for assistance.

REFERENCES

1. Artman, N.R., *Adv. in Lipid Res. 7*:245 (1969).
2. Potteau, B., and J. Causeret, *Rev. Fr. Corps Gras 18*:591 (1971).
3. Perkins, E.G., *Ibid. 23*:257 (1976).
4. Grandgirard, A., *Ann. Nutr. Alim 34*:377 (1980).
5. Causeret, J., *Cahier Nutr. Diet. 17*:19 (1982).
6. Márquez-Ruiz, G., M.C. Pérez-Camino and M.C. Dobarganes, *Grasas y Aceites 41*:432 (1990).
7. Billek, G., in *The Role of Fats in Human Nutrition*, edited by F.B. Padley and J. Podmore, Ellis Horwood, Chichester, 1985, pp. 163–172.
8. Causeret, J., B. Potteau and A. Grandgirard, *Ann. Nutr. Alim. 60*:483 (1978).
9. Lang, K., *Fette Seifen Anstrichm. 75*:73 (1973).
10. Rodríguez, A., C. Cuesta, F.J. Sánchez-Muniz and G. Varela, *Grasas y Aceites 35*:22 (1984).
11. Nolen, G.A., J.C. Alexander and N.R. Artman, *J. Nutr. 93*:337 (1967).
12. Billek, G., and G. Guhr, *Ernaehrung/Nutrition 3*:323 (1979).
13. Billek, G., *Nutr. Metabol. 24*:200 (1979).
14. Billek, G., G. Buhr and W. Sterner, *Fette Seifen Anstrichm. 81*:562 (1979).
15. Potteau, B., M. Lhuissier, J. Le Clerc, L. Custot, R. Mezonnet and R. Cluzan, *Rev. Fr. Corps Gras 17*:143 (1970).
16. Potteau, B., A. Grandgirard, M. Lhuissier and J. Causeret, *J. Bibl. Nutr. Dieta 25*:122 (1977).
17. Bottino, N.R., *J. Am. Oil Chem. Soc. 39*:25 (1962).
18. Friedman, L., W. Horwitz, G.M. Shue and D. Firestone, *J. Nutr. 73*:85 (1961).
19. Ohfuji, T., S. Iwamoto and T. Kaneda, *J. Japan Oil Chem. Soc. 19*:887 (1970).
20. Kajimoto, G., and K. Mukai, *Ibid. 19*:66 (1970).
21. Iwaoka, W.T., and E.G. Perkins, *J. Am. Oil Chem. Soc. 55*:734 (1978).
22. Márquez-Ruiz, G., M.C. Pérez-Camino and M.C. Dobarganes, *J. Chromatogr. 514*:37 (1990).
23. Márquez-Ruiz, G., M.C. Pérez-Camino, V. Ruíz-Gutiérrez and M.C. Dobarganes, *Grasas y Aceites 42*:32 (1991).
24. Metcalfe, L.D., and A.A. Schmitz, *Anal. Chem. 33*:363 (1961).
25. Long, T.T., L. Jakoi, R. Stevens and S. Quarfordt, *J. Lipid Res. 19*:872 (1978).
26. Miettinen, T.A., A. Proia and D.J. McNamara, *Ibid. 22*:485 (1981).
27. Hoet, P.P., J.V. Joosens, E. Evrard, H. Eyssen and P. De Somer, in *Biochemical Problems of Lipid*, edited by A.C. Frazer, Elsevier Publishing Company, Amsterdam, London, New York, 1963, pp. 73–83.
28. Márquez-Ruiz, G., M.C. Pérez-Camino and M.C. Dobarganes, *Fat. Sci. Technol.*, in press.
29. Frankel, E.N., *J. Sci. Food Agric. 54*:495 (1991).
30. Yagi, K., H. Ohkawa, N. Ohishi, H. Yamashita and T. Nakashima, *J. Appl. Biochem. 3*:58 (1981).
31. Sasaguri, Y., T. Nakashima, H. Morimatsu and K. Yagi, *Ibid. 6*:144 (1984).
32. Naruszewicz, M., E. Wozny, E. Mirkiewicz, G. Nowicka and W.B. Szoztak, *Atherosclerosis 66*:45 (1987).
33. Thomas, C.E., and R.L. Jackson, *J. Pharmacol. Exp. Ther. 256*:1182 (1991).
34. Lenz, M.L., H. Hughes, J.R. Mitchell, D.P. Via, J.R. Grytin, A.A. Taylor, A.M. Gotto and C.V. Smith, *J. Lipid Res. 31*:1043 (1990).
35. Kuzuya, M., M. Naito, C. Funaki, T. Hayashi, K. Asai and F. Kuzuya, *Biochim. Biophys. Acta 1096*:155 (1991).
36. Kanazawa, K., E. Kanazawa and M. Natake, *Lipids 20*:412 (1985).
37. Minamoto, S., K. Kanazawa, H. Ashida, G. Danno and M. Natake, *Agric. Biol. Chem. 49*:2747 (1985).
38. Kanazawa, K., and M. Natake, *Ibid. 50*:115 (1986).
39. Kanazawa, K., H. Ashida, S. Minamoto and M. Natake, *Biochim. Biophys. Acta 879*:36 (1986).
40. Ashida, H., K. Kanazawa, S. Minamoto, G. Danno and M. Natake, *Arch. Biochem. Biophys. 259*:114 (1987).
41. Ashida, H., K. Kanazawa and M. Natake, *Agric. Biol. Chem. 51*:2951 (1987).
42. Minamoto, S., K. Kanazawa, H. Ashida and M. Natake, *Biochim. Biophys. Acta 958*:199 (1988).
43. Bull, A.W., N.D. Nigro and L.J. Marnett, *Cancer Res. 48*:1771 (1988).
44. Bull, A.W., and J.C. Bronstein, *Carcinogenesis 11*:1699 (1990).
45. Bull, A.W., S.M. Earles and J.C. Bronstein, *Prostaglandins 41*:43 (1991).
46. Elitsur, Y., A.W. Bull and G.D. Luk, *Digestive Diseases and Sciences 35*:212 (1990).
47. Earles, S.M., J.C. Bronstein, D.L. Winner and A.W. Bull, *Biochim. Biophys. Acta 1081*:174 (1991).
48. Borgström, B., *Scand. J. Gastroenterol. 20*:384 (1985).
49. Tso, P., *Adv. in Lipid Res. 21*:143 (1985).
50. Carey, M.C., D.M. Small and C.M. Bliss, *Ann. Rev. Physiol. 45*:651 (1983).
51. Freeman, C.P., in *Proceedings of the 36th Nottingham Easter School*, edited by J. Wiseman, London, 1984, pp. 105–122.
52. Chang, S.S., and F.A. Kummerow, *J. Am. Oil Chem. Soc. 30*:403 (1953).
53. Williamson, L., *J. Appl. Chem. 3*:301 (1953).
54. Paulose, M.M., and S.S. Chang, *J. Am. Oil Chem. Soc. 50*:147 (1973).

[Received January 9, 1992; accepted June 25, 1992]