Occurrence of Cyclic Fatty Acid Monomers in Frying Oils Used for Fast Foods

E.N. FRANKEL, Northern Regional Research Center, Agricultural Research Service,
US Department of Agriculture, 1815 North University Street, Peoria, IL 61604; and
L.M. SMITH, C.L. HAMBLIN, R.K. CREVELING and **A.J. CLIFFORD**, Departments
of Food Science and Technology and of Nutrition, University of California, Davis, CA
95616

ABSTRACT

Cyclic fatty acid monomers were analyzed by gas chromatography in commercial frying oils obtained in this country and in the Middle East. Samples were obtained from food outlets in California and Illinois after varying periods of usage. The samples from Egypt and Israel were collected from street vendors frying vegetable patties (known as "fallafel") in open-air stands. The United States samples ranged from 0.1 to 0.5% cyclic monomers, and from 1 to 8% polar + noneluted thermal oxidation materials. The Middle Eastern samples showed significantly more heat abuse, with values for cyclic monomers from 0.2 to 0.7% and polar materials ranging from 2 to 22%.

INTRODUCTION

Cooking and deep-fat frying with polyunsaturated oils result in significant chemical changes that produce flavor deterioration in the fried foods and potential impairment in nutritional value. The literature in this field is extensive and several reviews have appeared (1-3). In the USA, soybean oil and other vegetable oils, intended for frying and other institutional uses, are generally hydrogenated to reduce most or all of the linolenic acid and a large portion of linoleic acid. However, in other countries and in the Middle East, many of the vegetable oils used for cooking and frying are not hydrogenated. If these oils are subjected to extensive heat abuse, their nutritional value may be damaged.

Changes occurring in heated oils have been followed by a large variety of analytical methods including acid, iodine and hydroxyl values, thiobarbituric acid tests, UV absorption, fatty acid composition, nonurea adduct-forming material, triglyceride content, noneluted material by gas chromatography (GC), polar-polymeric material by column chromatography, octanoate content, dielectric constant, color and viscosity (4-18). Much of the methodology reported for the analysis of thermal changes has been used without valid basis and may explain some of the controversy in the literature on the effect of heating unsaturated oils. Despite limitation of the methodology, there is now evidence that a measurable amount of cyclic monomers accumulate in heated vegetable oils (19,20).

No precise information has been available concerning which specific compounds are nutritionally harmful (1). Previous work of Iwaoka and Perkins (21) with rats showed that incorporation of 0.15% cyclic fatty acid methyl esters in low-protein diets containing 15% corn oil caused accumulation of liver lipids. Because of the markedly increasing consumption in this country of fried foods prepared in fast-food establishments, it is important to assess the amount of cyclic monomers actually formed in such commercial operations.

A GC procedure was previously developed (20) to determine quantitatively cyclic monomers formed in hydrogenated and unhydrogenated soybean oils heated at 195 C intermittently for 52 hr or continuously for 104 hr with frying of potato slices. In the present study, the same method for analyzing cyclic monomers was applied to a variety of samples of oils obtained from actual commercial frying operations. In the Middle East, vegetable patties known as "fallafel" are commonly deep-fat fried in vegetable oils by street vendors. Samples of oils used in such operations in Egypt and in Israel were obtained to compare their cyclic acid content with those from the USA.

EXPERIMENTAL

Samples

Fresh and used cooking oil samples were collected from several fast-food outlets in Peoria, Illinois, and in Davis, California, after varying periods of usage and just prior to being discarded. In the USA outlets, frying oil losses on foods were replenished daily. The types of foods fried included doughnuts, chicken, chicken liver, French fries, seafood, tofu and mixed substrates alternating between meat, pork cutlets and shrimp. Information on the number of frying cycles could not be obtained. History of usage of the Middle East oils was unknown. These Middle Eastern samples were collected at random from street vendors and transported to the USA by air. Length and conditions of usage varied widely. The Egyptian and Israeli samples were derived, respectively, from unhydrogenated cottonseed and soybean oils. All samples were stored under nitrogen at 4 C until analyzed.

Fatty Acid Composition

Major fatty acids were determined by GC of methyl esters prepared by the procedure of Metcalfe et al. (22). Instrumentation and conditions for GC included a Hewlett Packard Model 5711A chromatograph with flame ionization detector and a 610 × 0.257 cm id stainless steel column packed with 15% OV-275 (Supelco Inc., Bellefonte, PA) on high performance Chromosorb W. The column was operated isothermally at 220 C with injector and detector temperatures at 250 C. Nitrogen carrier gas flow rate was 10 mL/min. Standard mixtures of simple triglycerides were used to obtain relative retention times and response factors.

Cyclic Fatty Acid Monomers

The GC method used for cyclic monomers was that of Meltzer et al. (20). Briefly, the oil samples were saponified, hydrogenated and crystallized in acetone (−47 C) to remove 77-97% of the saturated linear fatty acids. The concentrated cyclic acids were then methylated and analyzed by GC using methyl heptadecanoate as an internal standard. The group of peaks assigned to cyclic monomers, which were eluted between methyl stearate and polar materials, was based on previous identification by comparison with a reference standard and by gas chromatography-mass spectrometry.

Thermal Oxidation Materials

Extent of oxidative degradation was estimated by measuring noneluted materials from a GC column according to the method of Waltking et al. (23). This procedure was adapted to methyl esters by using ca. 20% by weight methyl heptadecanoate as internal standard and by using their equation to calculate noneluted material.

TABLE I

Fatty Acid Composition (Weight Percent) of Fresh Cooking Fats[a]

Fatty acid	Vegetable shortenings (VS)[b]			Animal-vegetable shortenings (AVS)[c]				Partially hydrogenated vegetable oil (PHV)	Cottonseed oil (CSO)[d]		Soybean oil (SBO)
	D[e]	D	P[f]	D	D	P	P	P	D	E[g]	I[h]
12:0	–	–	1.3	0.1	0.1	–	0.1	0.1	–	–	–
14:0	0.1	0.1	0.6	3.7	3.5	3.9	4.3	0.3	0.8	0.8	0.1
16:0	11.4	11.0	15.2	26.8	26.4	27.7	27.2	13.5	20.3	23.0	7.4
16:1	0.2	0.2	–	5.5	5.2	–	–	–	0.7	0.6	–
18:0	11.8	13.0	9.8	18.1	18.8	21.1	22.9	9.8	2.8	2.8	5.4
18:1	74.0	72.3	70.6	42.6	41.7	44.9	42.4	45.6	19.6	19.5	26.8
18:2	2.3	2.9	2.5	2.4	3.4	2.4	3.1	29.9	55.0	52.8	53.6
18:3	0.2	0.2	–	0.5	0.6	–	–	0.8	0.3	0.5	6.7
20:1	–	0.3	–	0.3	0.3	–	–	–	0.5	–	–

[a] GC analyses are average of duplicate determinations.

[b] Hydrogenated soybean oil for "heavy-duty frying."

[c] Beef fats and cottonseed oil with dimethyl polysilane, BHA and BHT.

[d] Winterized cottonseed oil.

[e] Davis, CA.

[f] Peoria, IL.

[g] Egypt.

[h] Israel.

RESULTS AND DISCUSSION

Fats used by the restaurants sampled included vegetable shortenings (VS), animal-vegetable shortenings (AVS), partially hydrogenated vegetable oil (PHV) and cottonseed oil (CSO). Fats used in the Middle East included CSO in Egypt and soybean oil (SBO) in Israel. Fatty acid compositions were determined on the fresh unused fat sampled when available. Analyses in Table I show that monoenes vary from 46 to 74% and dienes from 2.3 to 29.9 in VS, AVS and PHV. In unhydrogenated SBO and CSO, the monoenes ranged from 20 to 27%, dienes from 54 to 55% and trienes from 0.3 to 7%. Only samples of AVS used were reported to include additives such as methyl polysilane, BHA and BHT.

GC analyses for cyclic monomers and polar + noneluted thermal oxidation materials varied widely with the origin, type and usage of the fats (Table II). The commercial USA samples ranged from 0.1 to 0.5% cyclic monomers and from 1 to 8% polar + noneluted materials. The corresponding fresh unused fats gave a value for materials with same GC retention ranging from 0.02 to 0.06% as cyclic monomers and from 0.6 to 1.9% as polar + noneluted materials. The lowest level of cyclic monomers was found in the fats used for doughnut frying (0.06-0.08%) and the highest level was found in the fats used for French fries (0.4-0.5%) and when CSO was used for frying (0.3-0.4%). The differences suggest that the cooking of French fries causes greater deterioration of the fats than cooking of the other foods. However, this assumes that comparable percentages of make-up fat were added daily to the different fryers used for each type of food.

The Middle Eastern samples showed significantly more heat abuse, with values for cyclic monomers from 0.2 to 0.7% and polar + noneluted materials from 2 to 22%. It is remarkable that, in all samples examined, the cyclic monomer content was less than 1%, even in the most abused samples that contained relatively large amounts of polar + noneluted materials. The low values for cyclic monomers may be accounted for by the dilution effect of make-up fat during the frying operation. No meaningful correlation was apparent between the level of cyclic monomer and polar + noneluted material.

These results confirm our previous study (20) with soybean oil and hydrogenated soybean oils heated in the laboratory under conditions analogous to those common in deep-fat frying operations. Although the level of cyclic monomer was in the same range (0.3-0.6%), the level of polar + noneluted material was generally lower in the commercial US samples (1-8%) than in the laboratory samples (18-21%). This difference would indicate a larger amount of thermal oxidation products in the laboratory samples. In another study, Guillaumin (24) showed the presence of cyclic monomers (0.07-0.2%) in all unsaturated fats examined by GC analysis. After 15 heatings at 200 C, cyclic monomers showed an increase of only 0.02-0.10%. To analyze for cyclic monomers, these workers used a direct gas chromatographic procedure without preliminary concentration (19). However, we found that at the low levels of cyclic monomers found in heated fat, it was necessary to concentrate the hydrogenated fatty acids prior to gas chromatography (20), and this observation was recently confirmed by Grandgirard and Julliard (25). Billek et al. (26) established an arbitrary level of 25-30% oxidized polar materials (by silica gel chromatography) to indicate that a frying oil is deteriorated to the discard point.

Caution must be exercised in interpreting the literature on nutritional effects of heated fats. Early reports (27-29) indicated that fats heated under frying conditions had no harmful effects. However, the diets used in these studies contained excessively high protein levels, which are known to lessen the effects of heated fats (30). In a more recent study, synthetic cyclic monomeric compounds, similar to those isolated from heated fats, have been shown to depress growth in rats and cause hepatomegaly or fatty livers due to accumulation of lipid (21). In this study, the diets were low in proteins to stress the animals, and the mineral mix used was deficient in important trace elements including zinc, cobalt, selenium and molybdenum. Additional data are needed on the level of cyclic monomers and polar materials in fats extracted from fried foods. Since pan frying is most commonly used in the world and may be more abusive to fats (24), further work is needed on the effect of this treatment on the extent of formation of oxidation product. Future work should also focus on the analysis of those polar and noneluted materials that were significant components of most of the heated samples examined in this work.

TABLE II

Cyclic Fatty Acid Monomers and Polar + Noneluted Materials in Commercial Cooking Oils[a]

Oil			Heating			Relative percent	
Origin[b]	Type[c]	Usage[d]	Temperature[e]	hr/day	Days	Cyclic monomers	Polar + noneluted
USA-D1	VS	Fresh	–	–	–	0.02	0.6
		Doughnuts	181	8	7	0.06	1.2
		Doughnuts	181	8	7	0.08	2.8
USA-D2	VS	Fresh	–	–	–	0.06	0.7
		Chicken	191	9	28	0.24	0.9
		Chicken livers	177	9	2	0.42	3.7
		French fries	177	9	2	0.40	3.7
		Chicken livers	177	9	2	0.37	1.3
		French fries	177	9	2	0.50	8.2
USA-P1	VS	Mixed	180	10	7	0.10	2.0
		Mixed	180	10	7	0.15	4.9
		Mixed	180	10	7	0.11	8.0
USA-P2	VS	Mixed	190	8	7	0.12	3.8
USA-D3	AVS	Fresh	–	–	–	0.06	1.1
		Seafood/ French fries	191	10	3.5	0.15	4.0
		Seafood/ French fries	191	10	7	0.14	2.3
USA-P3	AVS	Mixed	195	10	7	0.15	2.9
		Mixed	195	10	14	0.45	4.1
		Mixed	195	10	7	0.12	3.2
USA-P4	PHV	Fresh	–	–	–	0.03	1.9
		Mixed	190	8	7	0.34	4.2
USA-D4	CSO	Fresh	–	–	–	0.26	2.6
		Tofu	177	5	15	0.37	8.3
		Tofu	177	5	15	0.38	7.3
		Tofu	177	5	15	0.40	6.6
Israel	SBO	Fallafel				0.50	22.2
		Fallafel				0.44	2.3
		Fallafel				0.38	2.3
		Fallafel				0.20	4.5
Egypt	CSO	Fallafel				0.39	7.4
		Fallafel				0.66	10.1
		Fallafel				0.17	2.4
		Fallafel				0.48	9.9

[a] Method of Meltzer et al. (20).

[b] USA-D: Davis, CA; USA-P: Peoria, IL.

[c] See Table I; VS = vegetable shortening; AVS = animal-vegetable shortening; PHV = partially hydrogenated vegetable oil; CSO = cottonseed oil; SO = soybean oil.

[d] Mixed = mixture of substrates including meat, pork and shrimp. Fallafel = Middle Eastern vegetable patties—no exact history available on usage. Daily make-up with fresh oil in Davis and Peoria outlets.

[e] Temperature ± 5 C.

ACKNOWLEDGMENTS

Linda Parrott (NRRC) provided technical assistance in the GC analyses. P. Budowski (The Hebrew University, Rehovot, Israel) and S. El-Magoli (Cairo University, Cairo, Egypt) provided the Middle Eastern samples of heated fats.

REFERENCES

1. Artman, N.R., Adv. Lipid Res. 7:245 (1969).
2. Perkins, E.G., Rev. Fr. Corps Gras 23:257, 313 (1976).
3. Potteau, B., P. DuBois and J. Ricand, Ann. Technol. Agric. 27:655 (1978).
4. Sahasrabudhe, M.R., and V.R. Bhalerao, JAOCS 40:711 (1963).
5. Yuki, E., and Y. Ishikawa, JAOCS 53:673 (1976).
6. Perkins, E.G., and L.A. Van Akkern, JAOCS 42:782 (1965).
7. Krishnamurthy, R.G., T. Kawada and S.S. Chang, JAOCS 42:878 (1965).
8. Thompson, J.A., M.M. Paulose, B.R. Reedy, R.G. Krishnamurthy and S.S. Chang, Food Technol. 21:405 (1967).
9. Pardun, H., J. Blass and E. Kroll, Fette Seifen Anstrichm. 76:97 (1974).
10. Perkins, E., R. Taubold and A. Hsieh, JAOCS 50:223 (1973).
11. Billek, G., G. Guhr and J. Waibel, JAOCS 55:728 (1978).
12. Guhr, G., and J. Waibel, Fette Seifen Anstrichm. 81:511 (1979).
13. Fritsch, C.W., D.C. Egberg and J.S. Magnuson, JAOCS 56:546 (1979).
14. Graziano, V.J., Food Technol. 33:50 (1979).
15. Sagredos, A.N., Fette Seifen Anstrichm. 69:707 (1967).
16. Peled, M., T. Gutfinger and A. Letan, J. Sci. Food Agric. 26:1655 (1975).
17. Paradis, A.J., and N.W. Nawar, J. Food Sci. 46:449 (1981).
18. Peers, K.E., and P.A.T. Swoboda, J. Sci. Food Agric. 33:389 (1982).
19. Gente, M., and R. Guillaumin, Rev. Fr. Corps Gras 24:211 (1977).
20. Meltzer, J.B., E.N. Frankel, T.R. Bessler and E.G. Perkins, JAOCS 58:779 (1981).
21. Iwaoka, W.T., and E.G. Perkins, Lipids 11:349 (1976).
22. Metcalfe, L.D., A.A. Schmitz and J.R. Pelka, Anal. Chem. 38:911 (1966).

23. Waltking, A.E., W.E. Seery and G.W. Bleffert, JAOCS 52:96 (1975).
24. Guillaumin, R., Fette Seifen Anstrichm. 81:545 (1979).
25. Grandgirard, A., and F. Julliard, Rev. Fr. Corps Gras 30:123 (1983).
26. Billek, G., G. Guhr and W. Sterner, Fette Seifen Anstrichm. 81:562 (1979).
27. Poling, C.E., W.D. Warner, P.E. Mone and E.E. Rice, J. Nutr. 72:109 (1960).
28. Nolen, G.A., J.C. Alexander and N.R. Artman, Ibid. 93:337 (1967).
29. Poling, C.E., E. Eagle, E.E. Rice, A.M.A. Durand and M. Fisher, Lipids 5:128 (1970).
30. Witting, L.A., T. Nishida, O.C. Johnson and F.A. Kummerow, JAOCS 34:421 (1957).

[Received August 8, 1983]

Compositional Analysis of Natural Wax Ester Mixtures by Tandem Mass Spectrometry

GAYLAND F. SPENCER and **RONALD D. PLATTNER**, Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 1815 North University Street, Peoria, IL 61604

ABSTRACT

Tandem mass spectrometry is particularly suited for the analysis of complex, natural wax ester mixtures R_1-CO_2-R_2. Reduction of the mixture with deuterium provides species that are separable (through mass spectrometry) based on the number of original double bonds. Chemical ionization with isobutane produces high yields of protonated molecular ions and very little further fragmentation. These ions are separated by the first mass filter and then dissociated through collisions with argon. The positively charged dissociation products are almost exclusively the protonated acid ions $(R_1\text{-}CO_2H_2)^+$ that can then be separated by the second mass filter before detection and quantitation. The technique overcomes many of the obstacles previously faced during wax ester analysis. Results from this method are compared with those obtained by previous work, and the isomer composition of a new wax ester oil, orange roughy oil, is given.

INTRODUCTION

The analysis of naturally occurring wax ester mixtures for the relative abundances of isomers within each chain length is a somewhat formidable task. Since Aasen et al. showed that electron impact (EI) mass spectra could be used to quantitate saturated wax ester mixtures (1), mass spectrometry has become the method of choice of many workers (2-4). Complications encountered in these analyses include the difficulties associated with gas chromatographic separation of these relatively high molecular weight compounds and the problem of quantitatively introducing them into the mass spectrometer. Further, EI gave a great deal of nonspecific fragmentation with a relatively small percentage of the total ion current attributed to diagnostically important ions.

Recently, we showed that chemical ionization (CI) with isobutane gave spectra with intense protonated molecular ions and very little further fragmentation (5). Although this feature was not beneficial for structural information, it appeared to be particularly advantageous for mass spectrometry/mass spectrometry (MS/MS), because a high yield of ions representing the molecular species could be formed. When subsequent experiments showed that these ions could be dissociated to yield essentially one daughter ion per acyl radical (the protonated acid), a method was needed to identify and quantitate unsaturated isomers and analogs. Tris(triphenylphosphine)chlororhodium(I) catalyzes the reduction of double bonds with very little exchange between substrate and reagent (6), which results in saturates that include two atoms of deuterium per original double bond. Therefore, the protonated molecular ion and associated protonated acid ion from unsaturated compounds have m/z values 2 units greater per double bond and, although they behave chemically like their fully protonated counterparts, they are easily distinguished by mass spectrometry. Thus conditions were available to conduct the analysis in a single MS/MS experiment, because the entire wax ester mixture could be reduced with deuterium and the protonated molecular ions separated by the first mass filter. Following dissociation, the ions arising from the component acids could be analyzed in the second mass filter. In this paper we describe methods used to conduct such analyses and the results obtained from four natural wax ester mixtures.

EXPERIMENTAL

Purified wax ester standards were prepared from appropriate alcohols and acyl chlorides (2). Saponification (and recovery of unsaponifiables) was carried out essentially as prescribed by the AOCS (Method Ca 6b-53); the combined aqueous layers were then acidified and the free acids were

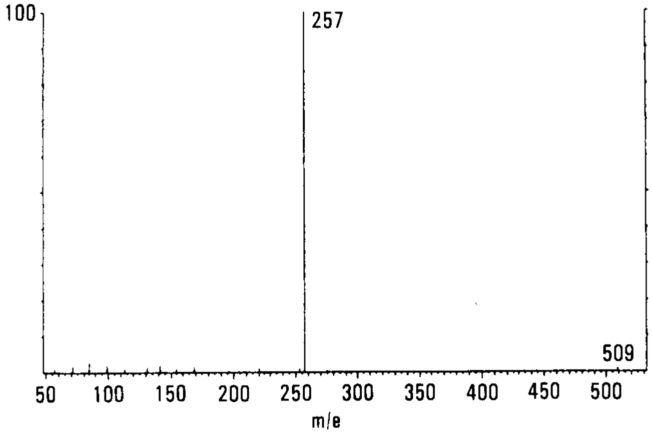

FIG. 1. Daughters of the protonated molecular ion from stearyl palmitate (18:0-16:0) produced by collision-induced dissociation with Ar; collision energy = −15 V.