Analysis of Thermally Abused Soybean Oils for Cyclic Monomers

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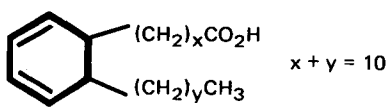
ABSTRACT

Cyclic monomers derived from the intramolecular condensation of the C_{18} polyunsaturated fatty acids have been reported to elicit toxic responses when fed to laboratory animals at low dietary levels. This study was undertaken to quantitate the cyclic monomers formed by thermal oxidation induced during deep fat frying to assess the potential toxicity of commonly used vegetable oils. Two separate experiments were designed to study the effects of unsaturation and both intermittent and continuous heating on cyclic monomer formation. Both lightly hydrogenated soybean oil (iodine value [IV] = 107) and refined, bleached and deodorized soybean oil were studied. The heated oil sustained substantial chemical and physical alterations, as indicated by IV decreases from 10-15 units, increases in free fatty acids of 5-10-fold and in noneluted material of 18-21%. Selected samples were completely hydrogenated and analyzed for cyclic monomers by gas chromatography. Under chromatographic conditions sufficiently sensitive to detect a cyclic monomer standard at less than 0.5% by weight, no cyclic monomers were detected in any of the heated oil samples. However, after concentration by low temperature crystallization of the hydrogenated samples to remove a major portion of the saturated components interfering in cyclic monomer resolution, about 0.3-0.6% cyclic acids, as well as 0.4-0.9% polar materials, were detected in the heated soybean oils. Components appearing in the gas chromatogram with the same retention time as those in a cyclic monomer standard were further identified by gas chromatography-mass spectrometry as disubstituted cyclic C-18 acids.

INTRODUCTION

Considerable attention has been directed toward the toxicity of heated oils during the last 30 years (1-3). Because of the continued popularity of foods prepared by deep fat frying, it has become important to establish limitations on the extended usages of frying oils for both quality control and consumer protection.

Cyclic monomers are included among the numerous types of nonvolatile oxidation products present in an abused oil. The most common monomers are the 18-carbon cyclic disubstituted fatty acids with the general structure:



These arise from the intramolecular condensation of the C_{18} polyunsaturated fatty acids. Several cyclic positional and configurational isomers have been isolated and their structures determined; the structures found were dependent on the conditions employed and the starting materials (4,5).

The early work of Crampton et al. (6,7) suggested a relationship between the nonvolatile, non-urea-adduct-forming fractions (NUAF) of abused oils and various toxic responses. Subsequent work confirmed these findings and identified the cyclic monomers, which constitute a major portion of the NUAF fraction, as the source of the toxicity (2,8-11).

Our previous studies have shown that small quantities (ca. 1%) of purified methyl ω-(2-alkyl cyclohexadienyl) carboxylic acids (cyclic monomers) produced decreased weight gains and feed consumptions in rats fed low levels of protein compared to control animals (12). Feeding the same levels of cyclic fatty acids resulted in altered lipogenesis in livers and adipose tissue in rats consuming either 8 or 10% protein diets (13).

Studies of cyclic monomer formation in vegetable oils have been confined to their generation by such treatments as alkali isomerization (14-16), metal catalysis (17) and thermal polymerization (18,19), none of which are representative of the thermal oxidative conditions existing during normal deep fat frying operations. Information on generation of cyclic monomers by thermal oxidation of linseed oil (5) is also irrelevant to deep fat frying with vegetable oils containing little or no linolenate. Gente and Guillaumin (20) heated vegetable oils including peanut, safflower, sunflower, soy and various mixtures of these oils in air at 220-240 C for 10 hr and detected cyclic monomers in the range of 0.15-0.55%. However, when these oils were used for frying potatoes at 180 C, cyclic monomers were in the range of 0.2-0.3%.

Although the French workers (20) claimed a limit of detection of 0.1% for cyclic monomers, in the present study it was necessary to concentrate the samples by low temperature crystallization to determine cyclic acids in oils at levels below 0.5%. This study was undertaken to quantitate the cyclic monomers formed in soybean oils under conditions specifically designed to be analogous to those common in deep fat frying operations with the aim of further assessing the food safety of heated oils and establishing limits on their extended use.

EXPERIMENTAL

Materials

Soybean oil (iodine value [IV] 129, Humko Products, TN) and partially hydrogenated soybean oil (IV 107, Anderson Clayton Foods, TX) were obtained through the generosity of the respective companies. They were refined, bleached and deodorized prior to shipment and stored in sealed containers at 32 F until used.

Preparation of Heated Oils

In one experiment, partially hydrogenated soybean oil was heated continuously for 104 hr, during which potato slices were fried at selected intervals. In the second and third experiments, partially hydrogenated and unhydrogenated soybean oils were heated intermittently for the same 104-hr period but heating was confined to the cooking periods only (52 hr).

In each experiment, 300 g of potatoes sliced in 1/4" thick discs were fried at 30-min intervals in 3 L of oil in a household model cooker-fryer containing oil maintained

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at 195 ± 5 C. A total of 72 lb of potatoes was fried over a period of 104 hr in each treatment. Samples were periodically removed, filtered, flushed with N_2 and refrigerated prior to analysis. Appropriate make-up oil was added to the fryer at the end of each day to replenish oil removed by sampling or absorption by the potatoes.

Preparation of Fatty Esters

Fatty acid methyl esters were prepared fromthe corresponding oils by heating samples of the oil with anhydrous methanol containing 2.5% sulfuric acid under reflux conditions. A reagent-to-sample ratio of 10:1 was employed. Upon cooling, the reaction mixture was extracted with both petroleum and diethyl ether until the extract was nearly colorless. The combined extracts were washed with water, dried over anhydrous sodium sulfate and the solvent removed under vacuum. Some samples were also transesterified with KOMe and the hydrogenated esters showed no difference in gas liquid chromatographic (GLC) analyses.

Hydrogenation of Methyl Esters

Fatty esters (50-100 mg) were catalytically hydrogenated over 100-200 mg platinum oxide catalyst in 15-20 mL ethyl acetate. Each reaction was allowed to proceed for 6 hr until no additional hydrogen uptake was observed. Catalyst and solvent were removed by filtration and evaporation, respectively. All samples were analyzed by GLC prior and subsequent to hydrogenation to ensure complete saturation.

Concentration of Samples

A method was developed to concentrate the hydrogenated cyclic monomers using Friedrich's (21) apparatus for low temperature crystallization. Heated oils were saponified by refluxing 15 min with 20% alcoholic KOH (10 g oil/20 mL reagent). The resulting fatty acids were hydrogenated for 7 hr over PtO_2 catalyst at atmospheric pressure and the hydrogenated acids were crystallized in acetone (-47 C) to remove 77-97% of the saturated fatty acids. These concentrated acids were then methylated with 0.4% H_2SO_4 in MeOH using the procedure already outlined.

GLC Analysis

Hydrogenated fatty esters were analyzed on a Hewlett-Packard Model 5830 A programmable gas chromatograph equipped with a flame ionization detector (FID) and electronic integrator. A glass column (6′ × 2 mm id) packed with 10% SP-2330 on 100/120 mesh Supelcoport (Supelco, Inc.) was used for analysis of extent of oxidation and cyclic monomers. The column oven temperature was programmed from 150-195 C at 4 C/min. The injection port and detector temperatures were 200 and 350 C, respectively. Carrier gas flow rate was 20 mL/min of nitrogen. All samples were dissolved in isooctane. Quantitation was achieved by using methyl pentadecanoate as an internal standard. AOCS standard fatty ester reference mixtures and a pure hydrogenated cyclic monomer standard synthesized by the methods of Eisenhauer et al. (22,23) were used to establish relative retention times

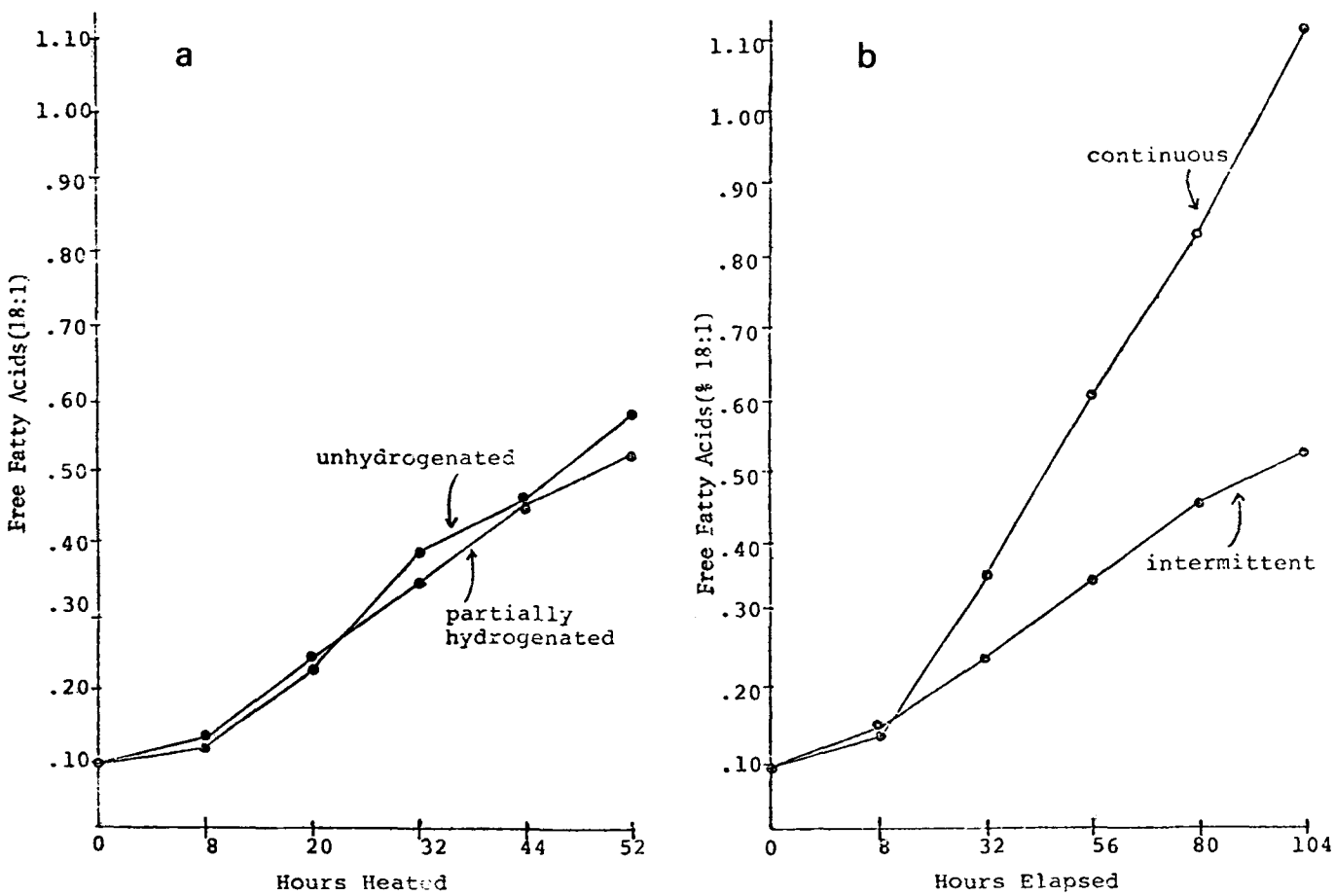

FIG. 1. (a) Effect of degree of unsaturation on free fatty acids of intermittently heated soybean oils; (b) effect of continuous and intermittent heating on free fatty acids of partially hydrogenated soybean oil.

under the conditions selected.

Concentrated samples obtained from low temperature crystallization were analyzed on a Hewlett-Packard Model 5710 A programmable gas chromatograph with FID. A 6′ × 1/8" id glass column containing the same column packing as already described was used. The column oven temperature was programmed from 180-210 C at 4 C/min. The injection port and detector temperatures were 250 and 300 C, respectively. Carrier gas flow rate was 20 mL/min of N_2; all samples were dissolved in CS_2. Quantitation was achieved by using methyl heptadecanoate as internal standard. As previously described (24), a computerized gas chromatography-mass spectrometry (GC-MS) system was used to identify cyclic acids in heated oils using as references synthetic saturated C_{18} cyclic monomers with either propyl or butyl substituents (25).

Characterization of Heated Oils

Free fatty acid (FFA) and IV were determined by AOCS standard procedures (26). Extent of oxidative degradation was estimated by measuring noneluted materials from a GLC column according to the method of Waltking et al. (27) after the fatty esters of the samples were fully hydrogenated.

RESULTS

Heating and degree of unsaturation were studied for their effects on the susceptibility of soybean oils to formation of cyclic monomers. Frying conditions were selected to simulate as closely as possible operations in small restaurants or homes. Thermal treatments were terminated when the oils appeared to have reached the ends of their useful lives as determined by excessive foaming.

FFA and IV determinations both find wide application in assessing thermal degradation of heated oils. Intermittent heating of both partially hydrogenated and unhydrogenated oils (Fig. 1a) resulted in the same FFA increase (ca. 5-fold). In contrast, continuous heating of partially hydrogenated oil caused a much greater FFA increase (ca. 10-fold) than intermittent heating (Fig. 1b), due to the difference in heating time. Intermittent heating of both partially hydrogenated and unhydrogenated oils resulted in IV decreases of ca. 10 units (Fig. 2a) whereas continuous heating of partially hydrogenated oil resulted in an IV decrease of ca. 15 units (Fig. 2b).

These two tests indicate that the heating treatments produced significant chemical changes in both partially hydrogenated and unhydrogenated oils. Changes in physical characteristics were assessed subjectively by observing increases in oil viscosities and colors, persistance of foaming and progressive product unacceptability with increasing cooking times.

The method initially selected for determining cyclic monomer content is dependent on complete hydrogenation of the fatty esters in the samples followed by GLC. While unsaturated cyclic monomer esters have GLC retention times similar to the unsaturated linear esters, hydrogenated cyclic esters can be separated from saturated linear esters of the same carbon number (22,23,27). During GLC of the hydrogenated sample, the components which eluted after methyl stearate but prior to methyl eicosanoate were considered to be the cyclic monomers on the basis of retention time. In this manner, cyclic monomer concentrations in the range of 0.1-50% have been determined (20,27). Preliminary experimentation with standard reference mixtures of linear esters indicated that a 2.5-min interval or "window" between these two components would provide adequate time for the separation of unsatu-

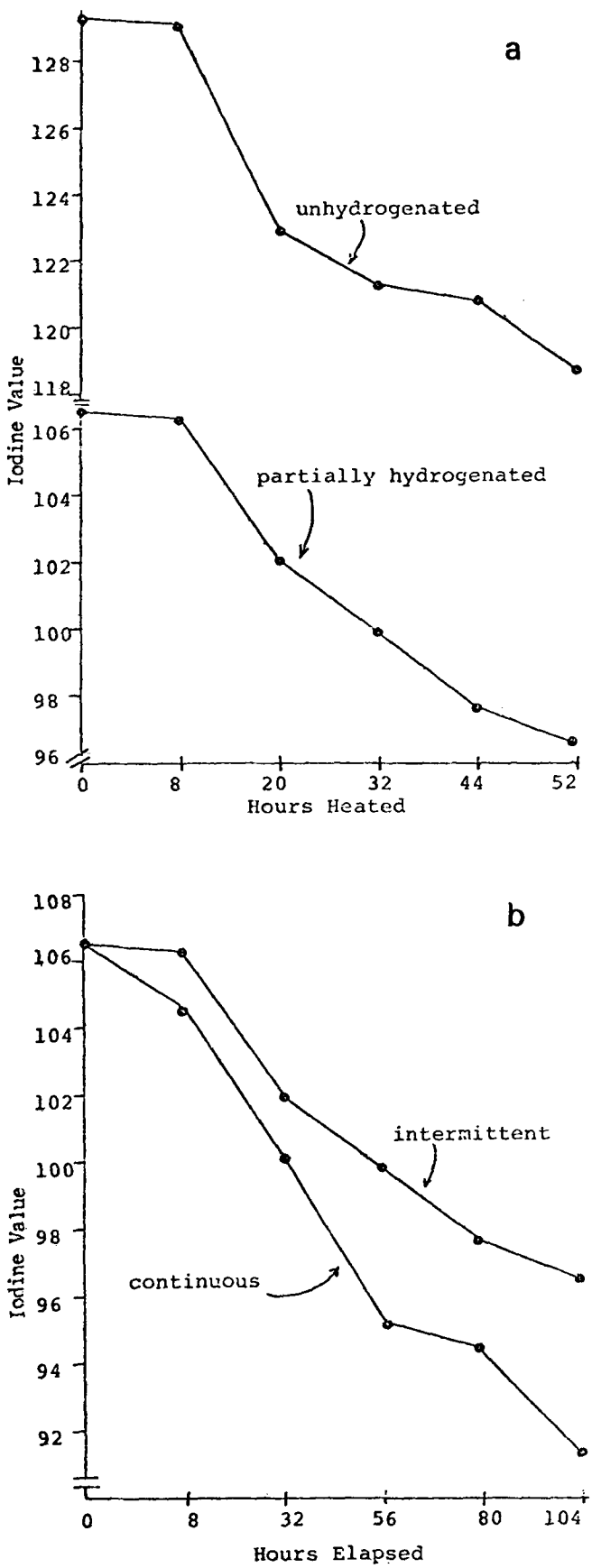

FIG. 2. (a) Effect of degree of unsaturation on iodine value of intermittently heated soybean oil; (b) effect of continuous and intermittent heating on iodine value of partially hydrogenated soybean oil.

rated C_{18} linear and cyclic monomer from methyl stearate and eicosanoate. GLC separation of pure hydrogenatd cyclic monomer standard resulted in nine peaks of area greater than 1%. Assuming that the same isomers present in the cyclic monomer standard would be found in hydrogenated esters from the heated oils, no attempt was made to further resolve these peaks.

Sensitivity of the chromatographic system for detecting cyclic monomers was established by spiking aliquots of the hydrogenated esters of the fresh oil with decreasing concentrations of the cyclic monomer standard until it could no longer be resolved from the stearate peak. The cyclic monomer standard could be detected and quantitated at a concentration of 0.5% by weight (Fig. 3). At a concentration of 0.1% by weight, the presence of the cyclic monomer was evident, however it was only partially resolved from the stearate peak as two shoulders and was too small to be recorded by the integrator.

Since unhydrogenated soybean oil was expected to be the most susceptible to monomer formation, the final sample of that oil was chromatographed at the same sensitivity at which 0.1% cyclic monomer could be seen but not quantified. There appeared to be a shoulder present on the tail of the stearate peak which could not be completely resolved.

Because no cyclic acids were detected by direct GLC analysis of the heated oils as hydrogenated fatty esters, a method was developed to concentrate the cyclic monomers through removal of a major part of the linear fatty acids from the hydrogenated sample by low temperature crystallization.

A model mixture of equal amounts of fully hydrogenated soybean esters and hydrogenated cyclic esters was subjected to GLC. Complete separation of the cyclic monomers from stearate was achieved with a 21′ column (Fig. 4a). The chromatograms obtained under the same conditions for the intermittently heated partially hydrogenated and unhydrogenated soybean esters after low temperature crystallization are shown in Figures 4b and 4c. Both oils displayed several peaks with retentions similar to those of the cyclic monomer reference. In addition to the cyclic monomer, the peaks included polar oxidation materials. Peaks corresponding to cyclic acids were further identified by GC-MS. Figures 5 and 6 show structural assignments of GC peaks made by computer plots of selected masses vs retention time (spectrum numbers). Cyclic acids with a propyl branch were thus identified by plotting characteristic masses 296 (M+), 253 (M-C_3H_7), 221 (253-CH_3OH), and 203 (253-CH_3OH-H_20). Fragment 189 corresponds to m/e 203 for cyclic acids with a butyl branch. The same mass fragments were previously reported for propyl and butyl branched cyclic acids as a major product of alkali and thermally cyclized linseed esters (5,25).

The results of quantitative GLC analysis of concen-

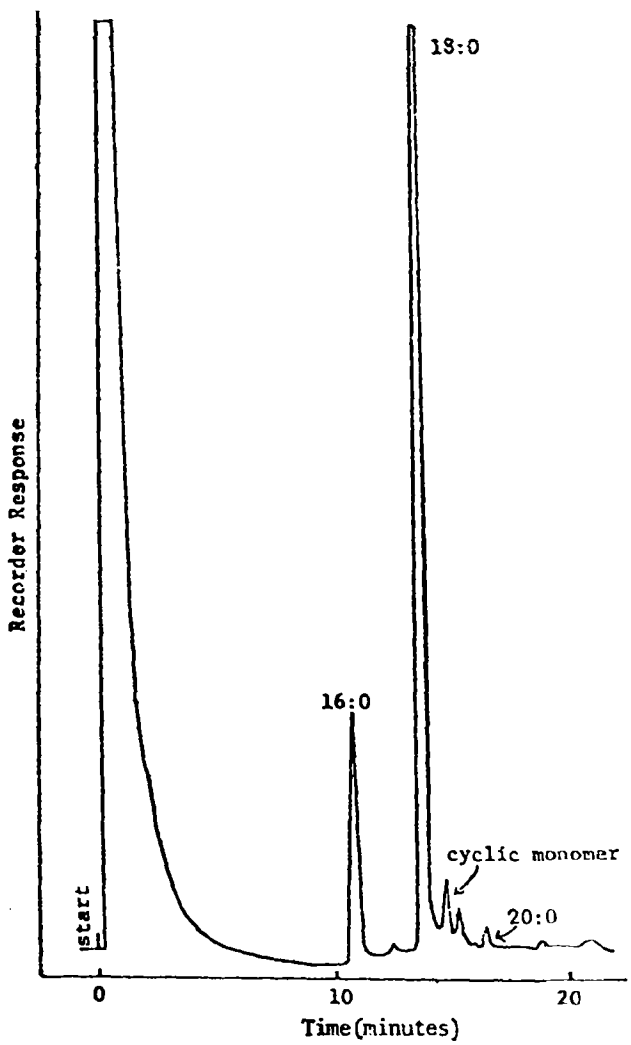

FIG. 3. Hydrogenated fatty acid methyl esters of unhydrogenated soybean oil spiked with cyclic monomer at 0.5% (w/w).

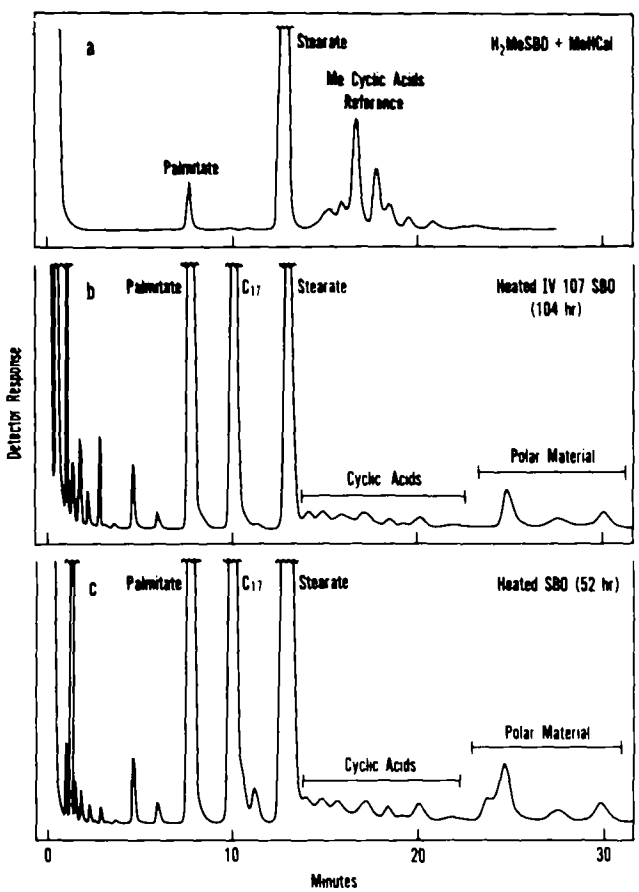

FIG. 4. (a) GLC of model system showing separation of stearate and cyclic acids; (b) GLC of heated hydrogenated soybean oil (IV = 107) after low temperature crystallization (c) GLC of heated soybean oil after low temperature crystallization (GLC conditions: 21′ × 1/8″ id glass column 10% SP-2330 on 100/120 Supelcoport, temp 1 = 180 C; temp 2 = 210 C; inj temp = 250 C; FID temp = 300 C; N_2 = 20 mL/min).

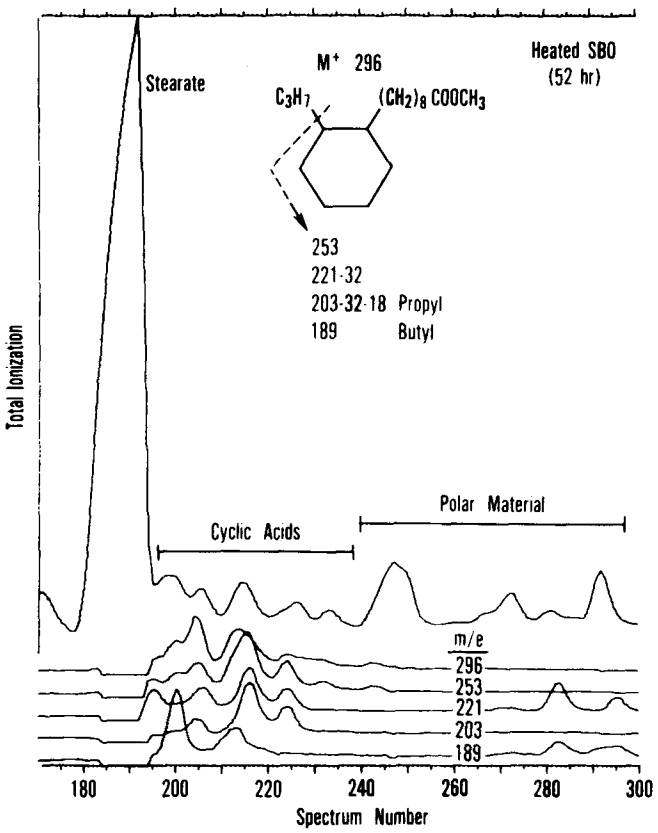

FIG. 5. GC-MS identification of cyclic C-18 acids in heated soybean oil (52 hr).

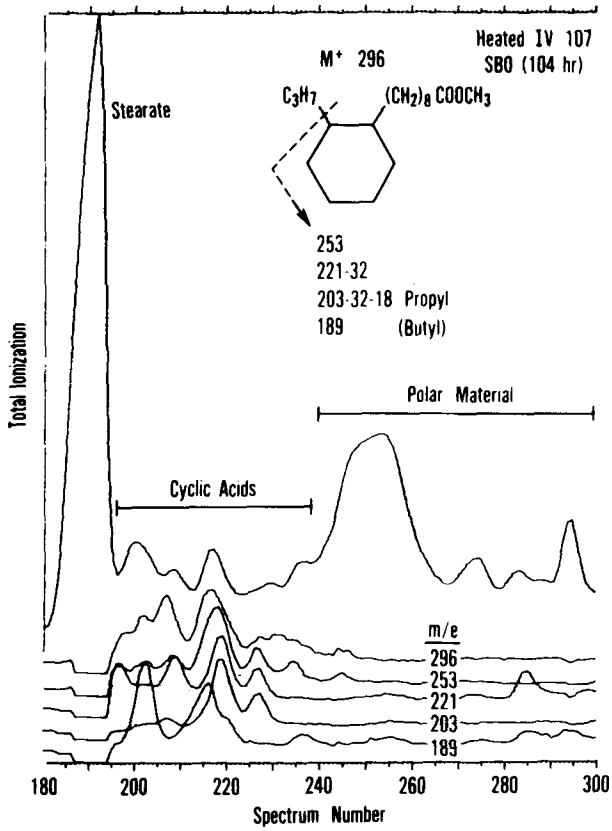

FIG. 6. GC-MS identification of cyclic C-18 acids in heated hydrogenated soybean oil (104 hr).

trated, heated oil samples are summarized in Table I. Increases in the amounts of noneluted materials reflect the presence of both polymeric and oxidative materials (28). The same amounts of noneluted material in the concentrated samples of the intermittently heated unhydrogenated and partially hydrogenated oils show comparable extents of thermal degradation. The continuously heated soybean oil shows the highest amount of noneluted material indicating the greatest amount of thermal deterioration. Values of 0.45% cyclic monomer were determined in the intermittently heated soybean oil, 0.33% in the intermittently heated hydrogenated oil, and 0.57% in the continuously heated hydrogenated oil. Concentrations of polar material followed the same trend as the noneluted material.

DISCUSSION

The formation of cyclic monomers in a vegetable oil depends on its degree of unsaturation and the temperatures to which it is exposed. Cleavage of oxidation products in an oil is competitive with both cyclization and polymerization. The formation of cyclic acids involves one molecule, whereas polymerization involves two or more molecules. Therefore, conditions which favor the development of one product should reduce the formation of the other (22). Thermodynamically, intramolecular condensation to form cyclic monomers is the most demanding of the oxidation changes occurring in a heated oil because it requires configuration changes prior to cyclization.

Recent work confirms that temperature is of primary importance; cyclic monomer formation does not occur to any significant extent until temperatures approach the thermal polymerization range of 200-300 C (5,25). Potteau et al. (5) found that thermal polymerization of linseed oil at 275 C (12 hr, N_2) produced 10 times more cyclic monomers than did thermal oxidation (200 C, 12 hr, air) of the same oil. Gente and Guillaumin (20) showed that the quantities of cyclic monomers formed in an abused oil are negligible until the initial linolenic acid content exceeds about 20%. However, Artman (2) suggested that oils containing fatty acids with higher degrees of unsaturation, such as linseed oil with 60% linolenic acid, have a more pronounced tendency to polymerize and form oxidative products than to cyclize intramolecularly during thermal oxidation in the range of 200 C.

Thus, the combination of less severe heating temperatures (180-190 C) and relatively lower concentrations of polyunsaturated fatty acids in oils used in deep fat frying operations must favor the formation of volatile and nonvolatile oxidation products resulting from decomposition of hydroperoxides, chain scissions and subsequent destruction of unsaturation, rather than cyclic monomers. As the double bonds are consumed by such competitive reactions, they will be unavailable for cyclization.

From the chemical assessments of the oils studied in these experiments, it is apparent that they sustained substantial oxidative degradation. Examination of the changes in the amount of material eluted from the column with increased heating indicates that significant oxidation and polymerization also occurred. The formation of hydroxy esters by saponification of the allylic hydroperoxides (29) would be expected to contribute to both polar and noneluted materials. Dimerization of hydroperoxides (30) would similarly be reflected in increased noneluted material. These phenomena appear to take place at the expense of cyclic monomer formation. GLC showed many minor peaks with retention times similar to those of the reference cyclic acids. These peaks were further identified by GC-MS

TABLE I

GLC Analysis of Heated Oils after Low Temperature Crystallization

Oils[a]	Treatment (195 C)	Cyclics[b]	Polar[b]	Noneluted Material[b,c]
SBO	52 hr, intermittent	0.454 ± 0.019	0.414 ± 0.009	18.4 ± 0.6
H_2SBO	52 hr, intermittent	0.332 ± 0.024	0.405 ± 0.025	18.3 ± 0.8
H_2SBO	104 hr, continuous	0.573 ± 0.090	0.910 ± 0.043	20.7 ± 0.4

[a]SBO: soybean oil, H_2SBO: hydrogenated to IV 107.

[b]Standard deviation estimated from 3 replicate analyses.

[c]Unheated oils contained 0.9-1.0% noneluted materials.

as due to disubstituted propyl and butyl branched cyclic C_{18} acids.

The extrapolation of these data generated by laboratory abuse of oils to actual commercial operations cannot be made until similar analyses on actual commercially abused oils are undertaken. Such a study would further resolve the current concern over the potential toxicity of heated oils due to cyclic monomers.

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