Mass Spectral Identification and Gas Chromatographic Determination of Chlorinated Bleaching Adducts in Flour-Containing Food Items†

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Bakery products and other flour-containing food items from the Food and Drug Administration's Total Diet Program were found to contain a series of unusual halogenated compounds when analyzed by a procedure designed for the gas chromatographic determination of chlorophenoxy alkyl acid herbicides as their methyl esters. These compounds were shown to occur in bleached flours and were not present in unbleached flours. Thus, they were assumed to be flour-bleaching adducts. Gas chromatography–mass spectrometry with chemical ionization (ethylene oxide) proved useful for the characterization of chlorinated derivatives of indigenous fatty acids: oleic, (*Z*)-9-octadecenoic acid, and linoleic, (*Z,Z*)-9,12-octadecadienoic, acid. Thus, 9,10-dichlorooctadecanoic acid, 9,10-dichloro-12-octadecenoic acid, 12,13-dichloro-9-octadecenoic acid, 9,10,12,13-tetrachlorooctadecanoic acid, 9-chloro-10-hydroxyoctadecanoic acid, 10-chloro-9-hydroxyoctadecanoic acid, isomers of chlorohydroxyoctadecenoic acid, and isomers of trichlorohydroxyoctadecanoic acid were identified and determined in several food items (breads, cakes, muffins, cookies, crackers, etc.). The most prominent residue was that of 9,10-dichloro–12–octadecenoic acid. Levels found in chocolate cake, yellow cake, and coffeecake commonly exceeded 20 ppm.

INTRODUCTION

The Food and Drug Administration (FDA) monitors residues of pesticides, industrial chemicals, metals, and nutrients in the nation's food supply, through several programs. Of these, the Total Diet Program is one of the FDA's oldest residue surveillance studies, having been in operation since 1964. Results of this study are published periodically (Gunderson, 1988). Extraction and cleanup procedures used routinely for pesticides and industrial chemicals are described elsewhere (Storrherr et al., 1971; Krause, 1980; Carson, 1981; FDA, 1981; Luke et al., 1981; Hopper, 1982; Luchtefeld, 1987; AOAC, 1990).

All food items are analyzed for the presence of chlorophenoxy alkyl acid herbicides. The herbicide analytes are extracted from an acidic solution, subjected to gel permeation chromatography (GPC) and Florisil column cleanup, and methylated just prior to GC determination. A series of unidentified analytical responses (UARs) eliciting responses from a halogen-specific GC detector have consistently been detected in flour-containing food items (e.g., cakes, breads, onion rings) analyzed according to this method. Since these food items have the common ingredient of flour, a survey of various types of flour was conducted. These UARs were found in all bleached flour samples, and none were present in unbleached flour.

The baking quality of wheat flours is enhanced with bleaching (the addition of oxidizing-maturing agents) which improves gluten strength and elasticity. High-ratio flours are formed by bleaching with chlorine or chlorine dioxide gases to produce a commodity that will absorb increased amounts of sugar and oil. Such flours find use in the manufacture of cakes and pastries (*Food and Nutrition Encyclopedia*, 1983).

For commercial flour (pH 6.2) 39 mg of Cl_2 was required to chlorinate 20 g of flour to a pH of 4.0. More than 98% of the Cl_2 reacted with organic compounds in the flour or was consumed by oxidation/chlorination reactions. Extraction of the treated flour showed that 27.0–33.7% of the total chlorine added was incorporated in the flour lipids (Wei et al., 1984). Chlorine adds to residues of unsaturated fatty acids in flour lipids. The levels of indigenous lipids in wheat flour, 16% oleic acid [(*Z*)-9-octadecenoic acid] (1), 63% linoleic acid [(*Z,Z*)-9,12-octadecadienoic acid] (2), and 5% linolenic acid [(*Z,Z,Z*)-9,12,15-octadecatrienoic acid] (3), were determined by gas chromatography. After treatment with chlorine (at 12.5 g/cwt) (275 ppm) values were 14, 47, and 3%, respectively (Coppock et al., 1960).

Unlike chlorine, chlorine dioxide (ClO_2) had no immediate effects on levels of unsaturated fatty acids in flour, even when treatment levels were elevated to 300 ppm (20 times the normal level) (Fisher et al., 1957; Gilles et al., 1958). However, Daniels et al. (1960) examined extracts of flour placed in storage for 39 days after being treated with similar excessive doses of chlorine dioxide. Levels of indigenous oleic, linoleic, and linolenic acids were diminished to 7.5, 14.5, and 0%, respectively. When this flour was stored under nitrogen, losses were much lower; e.g., linoleic plus linolenic acids decreased 71% in air vs 16% in nitrogen from the 5th to 12th day after treatment.

The above evidence suggests that the halogen-containing UARs discovered in flour-containing food items may have their origin in the flour-bleaching process. The current investigation was initiated with the intent of establishing the identity and confirming the source of these UARs and thereby aiding toxicologists in evaluating their significance in our nation's food supply.

EXPERIMENTAL PROCEDURES

Mass Spectrometry. Identification of these halogen-containing UARs was achieved through mass spectrometric (GC–MS) analysis performed on a VG 7070E-HF GC–MS interfaced to an 11-250 data system (VG Analytical, Wythenshawe, U.K.) and a Varian 3700 gas chromatograph. Ion source operating temperature was maintained at 250 °C with an ionizing voltage of 70 eV for electron ionization. Ethylene oxide-positive ion chemical ionization was performed at 200 °C and 45 eV. Wide-

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bore column analyses were accomplished on a DB-17 (15 m × 0.53 mm i.d.) fused silica column (J&W Scientific, Inc., Rancho Cordova, CA) interfaced through an open-split interface (SGE, Inc., Austin, TX) and using helium carrier gas (head pressure of 30 psi) and a column oven temperature of 200 and 240 °C. Low-resolution, EI and CI, full-scan mass spectra collected at a 1 s/decade scan rate were normalized after background subtraction.

Gas Chromatography. Quantification was accomplished with a DB-17 (30 m × 0.53 mm i.d.) fused silica column fitted in a Varian 3600 gas chromatograph with a halogen-specific (electroconductivity) detector. The flow rate of helium was 30 mL/min with a column temperature of 200 °C.

Synthesis of Methyl 9,10-Dichlorooctadecanoate (4). A solution of Cl_2 in CCl_4 (0.26 mmol/mL) was prepared by bubbling Cl_2 gas through 49.8 mL of CCl_4 to increase weight by 0.92 g. Methyl 1 (1.04 g, 3.51 mmol) was dissolved in 3 mL of CCl_4. In subdued light, and at 0 °C, 13.8 mL of Cl_2 in CCl_4 solution was added dropwise with stirring and allowed to react for 0.5 h after addition. Workup and removal of solvent (in vacuo) yielded 1.22 g (95%) clear, colorless, mobile oil.

Synthesis of Mixture of Methyl 9,10-Dichloro-12-octadecenoate (5a) and 12,13-Dichloro-9-octadecenoate (5b). Similar to the synthesis of 4, 0.99 g of methyl 2 (3.38 mmol) was dissolved in 2.5 mL of CCl_4 and cooled to −10 °C; 13.0 mL of a solution of Cl_2 in CCl_4 (0.26 mmol/mL) was cooled to −10 °C and added dropwise in subdued light with stirring. The mixture was allowed to react for 1 h after addition was complete. A yield of 1.14 g (93%) of clear, colorless, mobile oil remained after workup and removal of solvent in vacuo.

Synthesis of Methyl 9,10,12,13-Tetrachlorooctadecanoate (6). Similarly, 40 mL of Cl_2 in CCl_4 (0.26 mmol/mL) was added dropwise with stirring to 0.96 g of methyl 2 (3.26 mmol) dissolved in 2.5 mL of CCl_4. Reaction was carried out at room temperature in subdued light. The mixture was allowed to react for 2 h after addition was complete. In vacuo removal of solvent after workup yielded 1.35 g (95%) of clear, colorless, mobile oil.

Synthesis of Mixture of Methyl 9-Chloro-10-hydroxyoctadecanoate (7a) and Methyl 10-Chloro-9-hydroxyoctadecanoate (7b). Synthesis was performed in two phases. Epoxidation of methyl 1 was accomplished through adaptation of a previously described procedure (Swern, 1948). According to a process described by Swern et al. (1947), 25 mL of 0.215 N HCl in ethyl ether (5.37 mmol) was added dropwise to 0.90 g (2.88 mmol) of methyl 9,10-epoxyoctadecanoate (in subdued light) and allowed to reacted for 4 h. Workup produced 0.95 g (95%) of the chlorohydrin mixture, a colorless, mobile oil.

Synthesis of Mixture of Methyl Trichlorohydroxyoctadecanoates. The procedure adapted to the preparation of a mixture of methyl 9,10-epoxy-12-octadecenoate (8a) and methyl 12,13-epoxy-9-octadecenoate (8b) from methyl 2 is described elsewhere (Fieser and Fieser, 1967). Thus, to 1.12 g (3.61 mmol) of a mixture of 8a and 8b was added 30 mL of 0.215 N HCl in ethyl ether (6.44 mmol) dropwise in subdued light and with stirring. After 4 h, the solution was washed with water and dried and with additional workup produced 1.21 g (80.1%) of white solid.

Synthesis of Mixture of Isomers of Methyl Chlorohydroxyoctadecenoates. A mixture of epoxide isomers 8a and 8b was treated with HCl in ethyl ether as described by Swern et al. (1947) to form a mixture of the several isomers of methyl chlorohydroxyoctadecenoate.

RESULTS AND DISCUSSION

Electron ionization mass spectrometry of these UARs from flour-containing food items failed to produce evidence of a molecular ion. Spectra resembled those of methyl esters of long-chained, unsaturated fatty acids (e.g., methyl linoleate). Since little or no evidence of chlorinated fragments was observed and apparent molecular ions were not evident, conclusive structural data were not obtained. Lange (1986) reported the formation of intense protonated adduct ions through the use of ethylene oxide (oxirane) as reagent gas in the positive ion chemical ionization mass spectrometric analysis of unsaturated long-chain

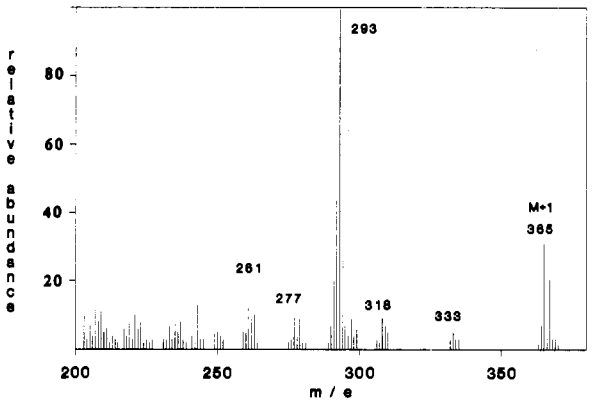

Figure 1. Chemical ionization mass spectrum (ethylene oxide) of methyl 9,10-dichloro-12-octadecenoate.

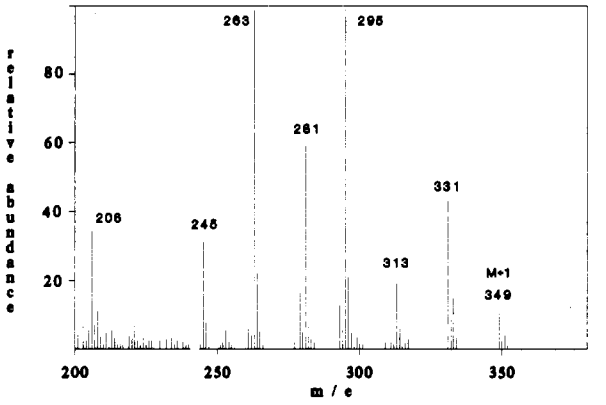

Figure 2. Chemical ionization mass spectrum (ethylene oxide) of methyl 9-chloro-10-hydroxyoctadecanoate.

hydrocarbons. This technique was applied to the UARs observed in bakery products and other flour-containing food items. The resulting spectra exhibited significant protonated molecular ion adducts with isotopic clusters indicative of one, two, three, or four chlorine atoms. Thus, evidence was accumulated for the existence of residues of 9,10-dichlorooctadecanoic acid (**9**), 9,10-dichloro-12-octadecenoic acid (**10a**), 12,13-dichloro-9-octadecenoic acid (**10b**), 9,10,12,13-tetrachlorooctadecanoic acid (**11**), 9-chloro-10-hydroxyoctadecanoic acid (**12a**), 10-chloro-9-hydroxyoctadecanoic acid (**12b**), isomers of chlorohydroxyoctadecenoic acid, and isomers of trichlorohydroxyoctadecanoic acid. The mass spectra of these methylated acids were similar to those of the synthesized methyl esters of chlorinated fatty acid compounds (e.g., Figures 1 and 2). Correlation was confirmed with both GC retention indices and mass spectral data, thus establishing identification.

The most abundant bleaching adducts determined as methyl esters in these table-ready foods were **10a** and **10b**, presumably formed from **2**, the dominant indigenous fatty acid in wheat flour. These isomers appear as two GC peaks with ratio a of 10:1, respectively, the most prominent being the energy-favored **10a**. Residues of **9** were found to be approximately 5% those of the combined amounts of **10a** and **10b**. All other chlorinated or chlorohydroxy derivatives of **1** and **2** were present at much lower levels. No evidence was seen to indicate the presence of chloro or chlorohydrin derivatives of **3**. It is suggested that experimental conditions may have been inadequate to chromatograph these less volatile compounds. Table I lists levels of **9** and the sum of **10a** and **10b** determined from food items of a recent market basket sample.

Komo-Suwelack et al. (1983) indicated that the pattern and composition of chlorinated bleaching products depend

Table I. Levels of 9,10-Dichlorooctadecanoic Acid [18:0 (Cl, Cl)] and Sum of Isomers of Dichlorooctadecenoic Acids [18:1 (Cl, Cl)] Determined in Food Items from a Recent Market Basket Sample

food item	C18:0 (Cl, Cl), ppm	C18:1 (Cl, Cl), ppm
cookies, chocolate chip	0.07	1.9
fish sticks, cooked	ND[a]	2.1
muffins, blueberry	0.14	2.6
saltine crackers	0.07	3.0
ice cream sandwich	0.15	3.9
white sauce	0.25	5.1
corn bread, southern	ND	5.7
onion rings	0.23	5.8
yellow cake	0.88	18
coffeecake	1.1	20
chocolate cake	1.4	26

[a] Not detected.

strongly on the amount of chlorine or chlorine dioxide applied to the flour. With an increase in the levels of chlorine, a decrease of mono and dichloro fatty acids, 18:1 (Cl, Cl) and 18:1 (Cl, OH), in favor of the corresponding saturated compounds, 18:0 (Cl, Cl, Cl, Cl) and 18:0 (Cl, Cl, Cl, OH), was observed. It was shown that chlorine bleaching can produce not only chlorinated fatty acids but also their chlorohydrin derivatives, while treatment with chlorine dioxide results in the production of only the chlorohydrins of **1** and **2**.

The toxicity of these chlorinated compounds has not been clearly established. A study by Daniels et al. (1963) involved a comprehensive multigeneration feeding of lipid from chlorine-treated flour to rats. No adverse reaction was noted at ingestion levels normally associated with consumption in the human diet. However, in a similar but more recent investigation, Cunningham (1980) demonstrated that chlorinated lipids in the diets of rats reduced weight gain and increased the size of the liver, kidney, and heart.

The method of analysis employed in this study was designed for the determination of chlorophenoxy alkyl acid herbicides and has not, as yet, been optimized for the determination of chlorinated bleaching byproducts. Future research will address this issue.

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Registry No. 1, 112-80-1; 1 methyl ester, 112-62-9; **2**, 60-33-3; **2** methyl ester, 112-63-0; **3**, 463-40-1; **4**, 33094-27-8; **5a**, 138834-41-0; **5b**, 138834-42-1; **6**, 33094-28-9; **7a**, 22348-93-2; **7b**, 10411-46-8; **8a**, 40707-88-8; **8b**, 18652-40-9; **9**, 5829-48-1; **10a**, 85556-75-8; **10b**, 85556-76-9; **11**, 26533-39-1; **12a**, 2632-61-3; **12b**, 2632-62-4; methyl trichlorohydroxyoctadecanoate, 138814-06-9; methyl chlorohydroxyoctadecenoate, 138876-05-8; chlorohydroxyoctadecenoic acid, 138834-43-2; trichlorohydroxyoctadecanoic acid, 138814-07-0.