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Chemical Ionization Mass Spectrometry of Fatty Acids: The Effect of Functional Groups on the CI Spectra[1]

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ABSTRACT

Chemical ionization (CI) mass spectra of functionally substituted fatty esters are a useful aid in determining molecular weight. Isobutane and ammonia CI mass spectra of various hydroxy, keto, epoxy and hydroperoxy fatty esters are reported and discussed.

INTRODUCTION

Although instruments with capabilities for chemical ionization mass spectrometry (CI-MS) have been widely available for a number of years, the literature is surprisingly sparse in systematic studies of CI mass spectra for fatty acids and their derivatives.

CI-MS has been used for the identification of polyunsaturated fatty acids in human serum (1). GC-MS with isobutane as the reagent gas yielded the quasimolecular ion $[M+H]^+$ as the base peak for methyl esters of fatty acids from C_{18} to C_{22} with up to six double bonds. Since the positions of the double bonds could not be deduced from the fragmentation of the underivatized fatty esters, the hydroxylated derivatives were analyzed by CI-MS. Fragments observed were similar to those found in electron impact mass spectrometry (EI-MS) of the same compounds. Suzuki et al. (2) reported CI-MS of methoxy derivatives of esters to locate double bonds in polyene fatty esters. CI-MS spectra of prostaglandins have also been reported (3). Stan and Scheutwinkel-Reich recently reported CI-MS data for the TMS derivatives of hydroxy fatty acids (4–6). They report that CI-MS is better than EI-MS because spectra show both position of OH substitution and molecular weight. Little additional information, however, exists in the literature for fatty acids with combinations of hydroxy, epoxy and/or keto groups in the molecular. We have undertaken a study of the CI mass spectra of these oxygenated fatty acids both as underivatized hydroxy or epoxy esters and as TMS derivatives or solvolyzed epoxide derivatives.

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Ion production by CI consists of reacting the substance under investigation with a known set of reactant ions. This is accomplished by operating the MS ion source at a relatively high pressure (on the order of 1 torr). The reactant ions are formed from the reagent gas by a combination of electron impact (EI) ionization and ion molecule reactions. The compound being analyzed is then chemically ionized within the same ion source by ion-molecule reactions. To suppress EI ionization, the concentration of the same is kept below $\sim$0.1% of the reagent gas concentration. The reactant ions may act with the sample as either Bronsted acids (by proton transfer) or as Lewis acids (by hydride abstraction), forming $(M+H)^+$ and $(M-H)^+$ ions. Additionally, the reactant ions may react with the sample to form a collision-stabilized complex $(M+\text{reactant ion})^+$ (7). These ionic species formed initially may have sufficient energy to further decompose, but the ionic species formed under CI have considerably less energy than the molecular ion formed under EI, and fragmentation is greatly reduced when compared to EI mass spectra. The initial ionic species is formed in a chemical ion-molecule reaction, and the nature of the product formed, its energy distribution, and the resulting fragmentation are jointly determined by the sample and the choice of reagent gas. Both ammonia and isobutane were investigated as reagent gas for fatty acids with various functional groups.

EXPERIMENTAL PROCEDURES

The mass spectra were obtained from a MS-30 mass spectrometer (Kratos Scientific Instruments, Westwood, NJ) equipped with a combined CI/EI source of a model 4535/TSQ quadruple mass spectrometer (Finnigan, MAT, Sunnyvale, CA). EI-MS were produced at 70 eV with a source pressure of approximately 10^{-6} torr on the MS-30, whereas CI-MS were produced at $\sim$150 eV with a pressure measured in the pumping housing arm of the MS 30 of 2×10^{-4} torr.

The MS source pressure for CI-MS was estimated to be ca. 0.5 torr. The 4535/TSQ source pressure was 0.3 torr as measured by the source pressure thermocouple gauge. Samples were introduced to the mass spectrometers by GC via a single stage jet separator or by direct insertion probe. Sample size for GC-MS analysis ranged from ca. 0.1 μg to 1 μg. Mass spectral data were acquired and analyzed with a DS 50S data system (Kratos) or Incos 2300 data system (Finnigan). Fatty acids analyzed were obtained commercially, synthesized by known procedures, isolated from seed oils or, where appropriate, separated from mixtures of methyl esters from such seed oils by GC. TMS derivatives were produced by heating 1–20 μg of the fatty methyl ester in 25 μL of hexamethyldisilazane/trimethylchlorosilane/pyridine (2:1:0.1) for 1 hr at 60 C in a screw-capped vial.

RESULTS AND DISCUSSION

CI-MS of methyl esters of fatty acids with only olefinic functionality yielded little information beyond the quasimolecular ion. With isobutane reagent gas, the protonated molecule accounted for nearly all of the ionization in methyl stearate, methyl linoleate and methyl linolenate. In methyl stearate, a small signal (∿7%) was observed at m/z 297 $(M-H)^+$ due to hydride abstraction from the molecular ion. Fragments of low intensity were observed at m/z 267 $[M+H-32]^+$, m/z 265 $[M-H-32]^+$ and m/z 74 and 87. When ammonia was the reagent gas there was even less fragmentation. In methyl stearate, the adduct ion $[M+NH_4]^+$ at m/z 316 was virtually the only significant signal in the spectrum. Inclusion of double bonds in the molecule increased the fragmentation slightly. However, the quasimolecular ion was still reported as the base peak for fatty esters, with up to six double bonds in isobutane CI (1). In ammonia CI we found $[M+NH_4]^+$ adduct was the base peak in the saturated, monoene, diene and triene esters. Tsang and Harrison (8) studied the effect of chain length on the CI spectra of saturated fatty esters from C_2 to C_{18} with CH_4 and H_2 reagent gases. They report that the amount of hydride abstraction increases, whereas the amount of protonation decreases with chain length for both H_2 and CH_4 reactant gases. However, the effect started to level out at higher chain lengths. We observed little variation in the $[M+H]^+$ and $[M-H]^+$ intensities observed with isobutane as the reagent gas from C_{16} to C_{22}.

When functional groups were added to the fatty ester molecule, the CI spectra became more complex. Addition of a hydroxyl group to the molecule produced a spectrum, with isobutane as reagent gas, with the $[M+H-H_2O]^+$ ion as the base peak. The isobutane CI-MS for methyl ricinoleate (12-hydroxy-*cis*-9-octadecenoate, Fig. 1a) showed weak ion intensities for $[M-H]^+$ and $[M+H]^+$ at m/z 311 and m/z 313. A fairly intense ion (m/z 263) was observed for the loss of methanol from the base peak of m/z 295. Table I lists the iosbutane CI spectra of several other hydroxy octadecanoates and hydroxy octadecenoates. The three isomeric monohydroxy octadecanoates (9-OH, 12-OH and 14-OH) all slowed small signals indicating the position of the OH group on the hydrocarbon backbone. In the 12-hydroxy octadecanoate these ions are at m/z 229, from cleavage alpha to the carbon atom with hydroxy group; m/z 197, arising from loss of methanol from m/z 229; and m/z 200, arising from cleavage of the $C_{11}-C_{12}$ bond with a hydrogen transfer. These ions are the same m/z values that are reported for EI-MS of hydroxy octadecanoates (9), but in the CI spectra they were much less intense.

In the ammonia CI spectra of methyl ricinoleate (Fig. 1b), the base peak at m/z 295 arises from the loss of water from the protonated molecular ion. However, unlike with isobutane, where the ion at m/z 313 $[M+H]^+$ is only 1% of the base peak, there is an intense ion (70% of base peak) at m/z 313. An intense ion is also observed at m/z 330 $[M+NH_4]^+$ (48% of base peak). The presence of ions, which represent the protonation and attachment of NH_4^+ to the molecule, was typical of the spectra of fatty esters with oxygenated functions. The loss of water from the protonated molecule with isobutane usually yields an intense

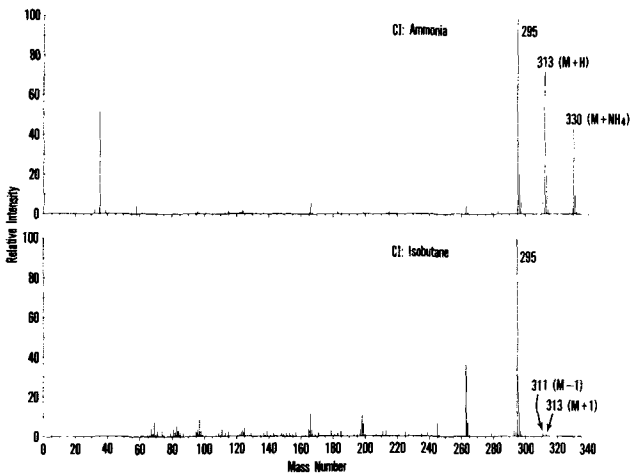

FIG. 1. Chemical ionization mass spectrometry of methyl ricinoleate.

TABLE I

Isobutane CI Spectra of Hydroxy Fatty Acid Methyl Esters

Compound	MW	Base peak	Other important ions
Methyl 9-hydroxyoctadecanoate	314	297 [M+H-18]	315 [M+H] (5), 187 (1), 158 (1), 155 (2)
Methyl 12-hydroxyoctadecanoate	314	297 [M+H-18]	315 [M+H] (5), 229 (1), 200 (1), 197 (2)
Methyl 14-hydroxyoctadecanoate	314	297 [M+H-18]	315 [M+H] (5), 257 (2), 228 (1), 225 (1)
Methyl 12-hydroxy-*cis*-9-octadecenoate (methyl ricinoleate)	312	295 [M+H-18]	313 [M+H] (1), 311 [M-H] (2), 263 (40)
Methyl 14-hydroxy-*cis*-11-eicosenoate	340	323 [M+H-18]	341 [M+H] (1), 339 [M-H] (2), 291 (46)
Methyl 9,10-dihydroxy octadecanoate	330	313 [M+H-18]	387 (M+57] (tr), 331 [M+H] (5), 329 [M-H] (3), 295 [M+H-2 X 18] (55), 281 [M+H-(18+32)] (90), 263 [M+H-(2 X 18+32)] (20), 187 (10), 171 (8), 155 (9)
Methyl 12,13-dihydroxy.octadecanoate	330	313 [M+H-18]	387 [M+57] (tr), 331 [M+H] (7), 328 [M-H] (1), 295 (20), 281 (90), 263 (15), 229 (10), 213 (7), 197 (8)

TABLE II

Ammonia CI Spectra of Hydroxy Fatty Acid Methyl Esters

Compound	MW	Base peak	Other important ions
Methyl 12-hydroxyoctadecanoate	314	297 [M+H-18]	332 [M+NH_4] (85), 315 [M+H] (20), 314 [M] (55), 246 (6), 214 (11), 200 (3)
Methyl 12-hydroxy-*cis*-9-octadecenoate (methyl ricinoleate)	312	295 [M+H-18]	330 [M+NH_4] (43), 313 [M+H] (20), 312 [M] (70), 213 (4), 166 (6)
Methyl 14-hydroxy-*cis*-11-eicosenoate	340	340 [M or M+NH_4-18]	358 [M+NH_4] (56), 323 (9), 307 (4), 291 (6), 194 (9)
Methyl 9,10-dihydroxy octadecanoate	330	348 [M+NH_4]	330 [M^+ or M+NH_4-18] (30), 318 (18), 295 (4), 204 (12)

fragment, sometimes nearly excluding the M+H ion. With ammonia, either $[M+H]^+$, M^+ or $[M+NH_4]^+$ ions may be observed; therefore, the determination of the molecular weight of an unknown is not always straightforward. Often in ammonia CI spectra of hydroxy acids, signals for both $[M+NH_4]^+$ and M^+ are observed. It is not readily apparent whether the signal at M^+ is a molecular ion or whether it is a loss of water from the adduct ion. Table II lists the ammonia CI spectra of several hydroxy fatty esters.

Both the isobutane and ammonia CI spectra of dihydroxy fatty ester isomers showed only molecular weight information. The position of the hydroxyl groups was not detected among the other low-intensity ions that were observed (see data in Tables I and II). Better chromatographic behavior is obtained for polyhydroxy fatty acids when they are derivatized to increase volatility. Fortunately, the TMS derivative generally chosen to improve GC characteristics of polyhydroxy fatty acids also improves the mass spectra obtained, by facilitating cleavages at the backbone on both sides of the trimethylsilyloxyl group. The intense backbone α cleavages for TMS derivatives of hydroxy fatty acid methyl esters have been used to determine the position of functional groups by EI-MS (10). In CI, the TMS derivatives of hydroxy fatty esters did not yield α cleavage ions of great intensity (they are the base peaks in EI-MS), but medium intensity ions were observed for these cleavages indicating functional group position. The base peak in the isobutane CI mass spectrum of 12-OTMS octadecanoate (Fig. 2a) was at m/z 297, arising from loss of HOTMS (90) from the protonated molecular ion. Small signals (3–5% of base peak) were observed for $[M+H]^+$, $[M-H]^+$, $[M+H-15]^+$ and $[M-H-15]^+$. As reported by Stan et al. (4, 5), the two α cleavage ions locating the position of the OTMS group on the backbone at m/z 301 and m/z 187 were 10–15% of the base peak. Similar α cleavage ions were observed in the other isomers (see Table III). The base peak in the ammonia CI mass spectrum of 12-OTHS octadecanoate (Fig. 2b) was at m/z 387 $[M+H]^+$. The adduct ion at m/z 404 $[M+NH_4]^+$ was 12% of the base peak. Intense ions

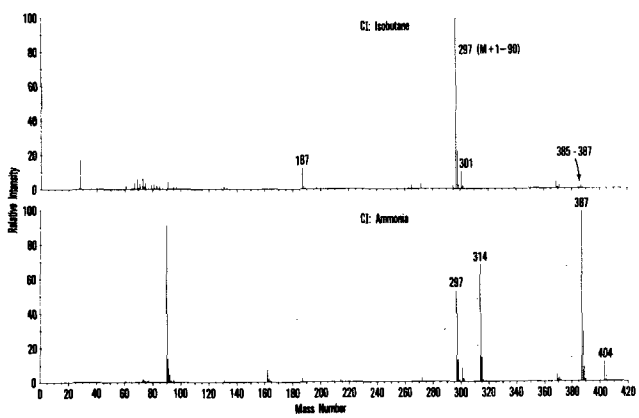

FIG. 2. Chemical ionization mass spectrometry of methyl 12-OTMS octadecanoate.

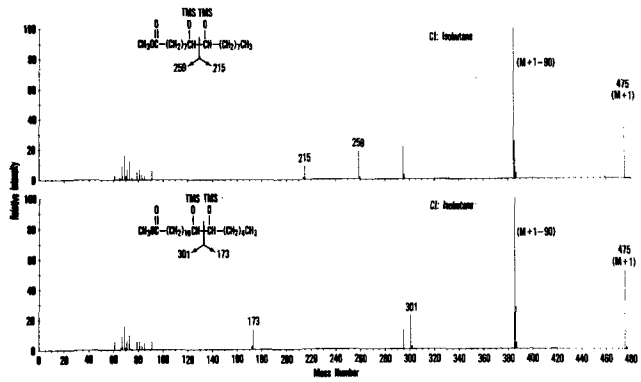

FIG. 3. Chemical ionization mass spectrometry TMS derivatives of isomeric dihydroxyoctadecanoates.

TABLE III

Isobutane CI Spectra of TMS Derivatives of Hydroxy Fatty Esters

Compound	MW	Base peak	Other important ions
Methyl 12-OTMS octadecanoate	386	297 [M+H-90]	387 [M+H] (3), 385 (3), 371 (4), 369 (5), 301 (12), 187 (14)
Methyl 9,10-diOTMS octadecanoate	474	385 [M+H-90]	475 (M+H] (2), 469 (1), 295 [M+H-2 X 90] (23), 259 (21), 215 (10)
Methyl 12,13-diOTMS octadecanoate	474	385 [M+H-90]	475 [M+H] (40), 295 (15), 301 (22), 173 (11)
Methyl 9,10,12-triOTMS octadecanoate	562	473 [M+H-90]	573 [M+H] (63), 383 (M+H-2 X 90) (31), 361 (10), 359 (30), 332 (10), 293 (5), 259 (20), 187 (45)

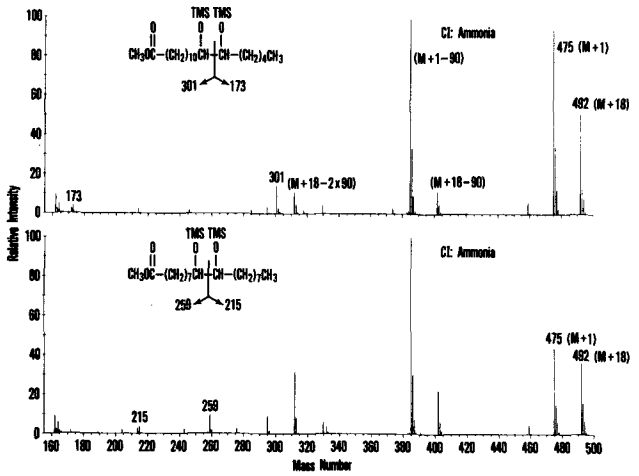

FIG. 4. Chemical ionization mass spectrometry of isomeric di-OTMS octadecanoates.

were also observed at m/z 314 $[M+NH_4-90]^+$ and m/z 297 $[M+H-90]^+$ and m/z 90. The α cleavage ions similar to those reported for isobutane CI (4, 5) were observed at m/z 301 and m/z 187. Figures 3 and 4 show the CI mass spectra of the TMS derivatives of isomeric dihydroxyoctadecanoates using isobutane and ammonia as the reagent gases, respectively. Again, α cleavage fragments identifying the positions of the hydroxyl groups were clearly evident, although unlike the EI spectra they are not the base peaks in the spectra. However, unlike EI-MS, when more than one OTMS group is in the molecule, the isobutane CI mass spectra always showed intense ions from protonated molecules. With ammonia, both the adduct $[M+NH_4]^+$ and $[M+H]^+$ ions were present. This makes CI mass spectra of TMS derivatives of polyhydroxy fatty esters more useful than their EI counterparts in the analysis of complex mixtues, such as lipid oxidation products, when the molecular weight of the components may be in question (5).

Figure 5 shows the EI and isobutane CI spectra of methyl 13-ketooctadecanoate. The protonated molecule (m/z 313) was the base peak in the CI spectrum. The other intense peak at m/z 281 arises from loss of methanol from

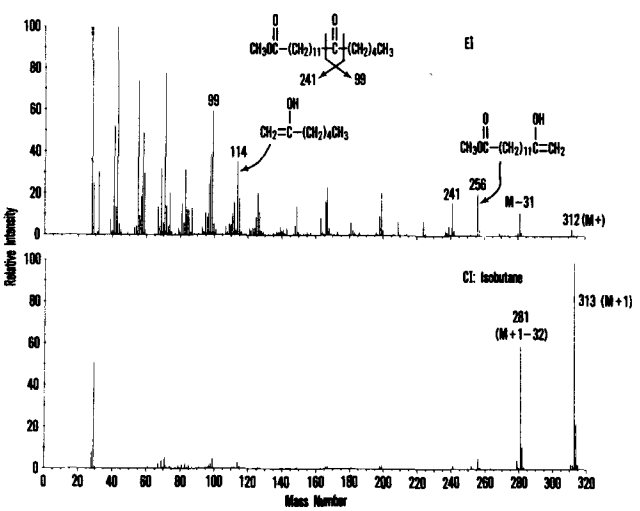

FIG. 5. Mass spectra of methyl 13-ketooctadecanoate.

$[M+H]^+$. Ions useful to locate the keto group in the EI spectrum (9) occur at much lower intensity in the CI spectrum. Under ammonia CI conditions, the base peak in the spectrum of methyl 13-ketooctadecanoate was at m/z 330 $[M+NH_4]^+$. The $[M+H]^+$ peak at m/z 313 was 70% of the base peak, and small signals (<4%) that locate the keto group also occurred at m/z 99 and 241 (Fig. 5).

Epoxy fatty esters gave EI spectra that showed extensive fragmentation, with little or no indication of molecular weight or the position of the epoxide group. Consequently, Kleiman and Spencer showed that the position of the epoxide group could be determined by converting the epoxide to a hydroxy-methoxy group by solvolysis of the epoxide ring with BF_3-methanol (10), followed by GC-MS as a TMS derivative. As Figure 6 shows, the isobutane CI spectrum of methyl 12,13-epoxyoctadecanoate is more useful than the EI spectrum. Under isobutane CI conditions, the protonated molecule m/z 313 occurred as a respectably large signal of 40% of the base peak at m/z 281, $(M+H-32)^+$. Other intense ions were observed at m/z 295 $[M+H-18]^+$ and m/z 263 $[M+H-18+32]^+$. Significant fragments at m/z 197, 213 and 229 arise from rearrangements involving loss of the epoxide at C_{12} and C_{13}. In methyl 9,10-epoxyoctadecanoate, the analogous fragments are observed at m/z 155, 169 and 187, whereas in methyl vernolate (methyl 12,13-epoxy-*cis*-9-octadecenoate) they are at m/z 195, 211 and 227 (see Table IV). Solvolysis of the epoxide with

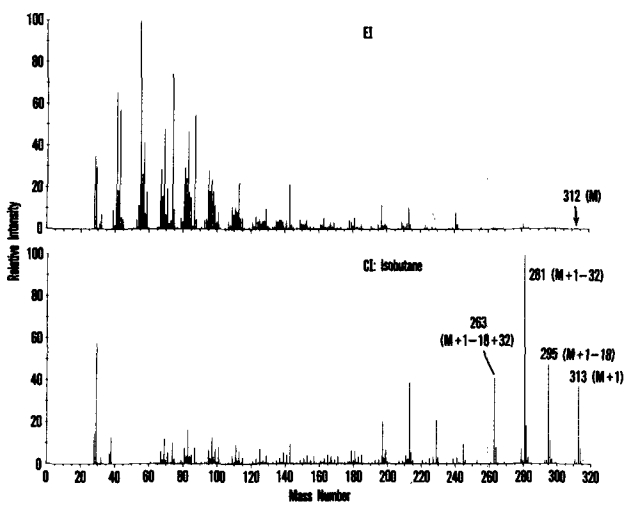

FIG. 6. Mass spectra of methyl 12,13-epoxyoctadecanoate.

BF_3 methanol to a methoxy-hydroxy derivative, or to a dihydroxy derivative followed by CI GC-MS of the TMS derivative is a better procedure for locating the position of an epoxide group because of the straightforward interpretation of the α cleavages previously discussed for hydroxy fatty acids.

We have found CI-MS especially useful when examining isomeric mixtures of oxygenated fatty acids, such as derived from the free radical decomposition of linoleic acid hydroperoxide. A complex mixture of products is resistant to complete GC-MS analysis unless it is subjected to a prior purification by chromatographic methods. Under EI conditions, these compounds yield spectra that often do not reveal any reliable indication of the molecular weight, making structure assignments of GC peaks quite difficult. Furthermore, in these complex mixtures, isomers are incompletely resolved by GC, and the assessment of the purity of a GC component by EI-MS is virtually impossible

TABLE IV

Isobutane CI Spectra of Epoxy Fatty Esters

Compound	MW	Base peak	Other important ions
Methyl 12,13-epoxyoctadecanoate	312	281 [M+H-32]	313 [M+H] (43), 295 [M+H-18] (50), 263 [M+H-18+32] (45)
Methyl 9,10-epoxyoctadecanoate	312	281 [M+H-32]	313 [M+H] (40), 295 [M+H-18] (48), 263 [M+H-18+32] (43)
Methyl 12,13-epoxy-*cis*-9-octadecenoate	310	279 [M+H-32]	311 [M+H] (99), 293 [M+H-18] (63), 261 [M+H+18+32] (7)
Methyl 9,10-*trans*-epoxy-13 keto-*trans*-11-octadecenoate and methyl 12,13-*trans*-epoxy-9-keto-*trans*-11-octadecenoate	324	325 [M+H]	309 [M+H-16] (10), 307 [M+H-18] (12), 293 [MH-32] (30), 185 (7), 99 (4)

because of extensive fragmentation. For example, the ferrous (cysteine-$FeCl_3$) catalyzed decomposition of linoleic acid hydroperoxide formed a mixture of ketodienes, trihydroxymonoenes, hydroxyketomonoenes, epoxyketoenes and epoxyhydroxyenes (11). EI-MS analysis of the TMS/methyl ester derivatives of this reaction mixture yielded a complex pattern of overlapping peaks. The TMS epoxyhydroxyene isomers can be identified by the intense fragments that arise from α cleavage to the OTMS group. The ketodienes and epoxyketomonoene isomers were not so simply identified, because they have neither intense fragments nor molecule ions in EI-MS. However, CI-MS yielded intense ions at MH^+ or $M+H^+-H_2O$ to indicate molecular weight for these compounds. Overlapping GC peaks of these isomers with hydroxy (TMS) functionality became apparent. Figure 7 shows a portion of the reconstructed ion chromatogram of isobutane GC CI-MS of the above mixture. Single ion chromatograms of m/z 399 $[M+H]^+$ and 309 $[MH-90]^+$ show the location of the methyl epoxy-OTMS-octadecenoate isomers. The ketodiene isomers are indicated by m/z 309 $[M+H]^+$ in the absence of m/z 399. The chromatogram of m/z 325 $[M+H]^+$ locates the methyl epoxyketooctadecenoate isomers. One isomer of the epoxyketoene coeluting with the epoxyhydroxyene was overlooked by EI-MS of the reaction mixture because no intense fragments were apparent to identify it.

CI-MS also is useful for examining the intact hydroperoxides without derivatization by direct probe insertion. Figures 8 and 9 show the isobutane and ammonia CI spectra of methyl 9-hydroperoxy-*trans*-10,-*cis*-12-octadecadienoate. The isobutane reagent gas yielded small signals at m/z 327 $(M+H)^+$ and 325 $(M-H)^+$, and larger signals were at m/z 311 $(M+H-O)^+$ and m/z 309 $(M-OH)^+$. A large peak at m/z 293 arises from loss of HOOH from the protonated molecular ion. In the ammonia CI spectrum, a signal at m/z 344 originated from attachment of NH_4^+ to the molecule. This ion lost oxygen to yield the base peak at m/z 328. Additional ions were observed at m/z 311, 309 and 293. Small differences between the spectra of the 9 and 13 hydroperoxy isomers were observed (data not shown). MS/MS of daughter fragments from the CI spectra of the isomeric 9 and 13 hydroperoxides is more useful in establishing the location of the peroxide. These studies currently are underway and will be reported separately.

CI mass spectra will prove to be an increasingly useful tool in oxidation studies. Because there is substantially less ionization energy in CI, the compounds fragment less and determination of molecular weight is simplified. Diagonistically important fragments arising from backbone cleavage are usually weaker than the corresponding EI spectra, thus CI spectra should be obtained in conjunction with EI spectra to obtain maximum information. The nature of the fragmentation that does occur in CI depends on both the chemical nature of the compound and the reagent gas (as well as the operating conditions), so interpretation of CI data must rely heavily on experience with model compounds and the chemical intuition of the investigator.

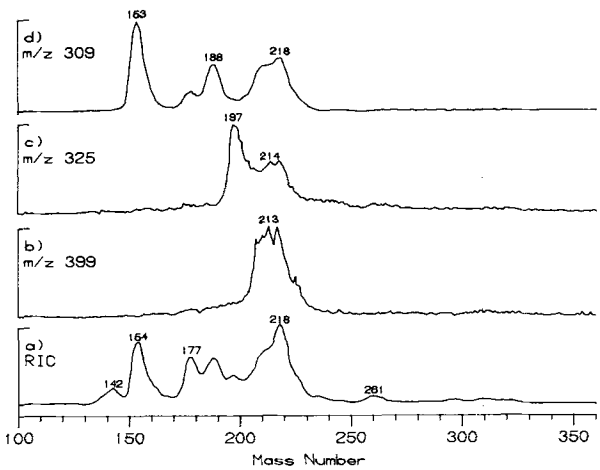

FIG. 7. Partial chromatogram of various products of methyl ester/TMS derivative from free radical decomposition of 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoic acid. (a) m/z 309 chromatogram (MH^+ for ketodienes and MH-90 for epoxyhydroxyene isomers); (b) m/z 325 chromatogram (MH^+ of epoxyketoene isomers); (c) m/z 399 chromatogram (MH^+ of epoxyhydroxyene isomers); and (d) reconstructed total ion chromatogram.

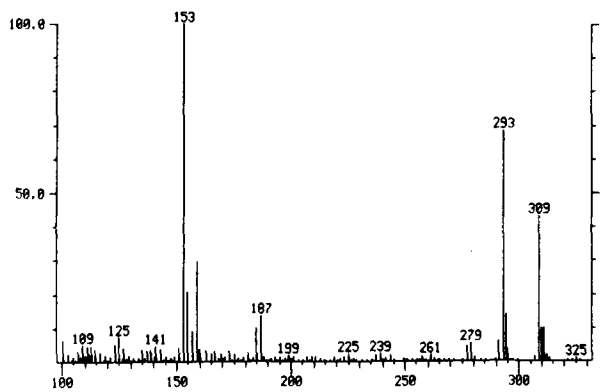

FIG. 8. Isobutane CI-MS of methyl 9-hydroperoxy-*trans*-10,-*cis*-12-octadecadienoate by direct insertion probe.

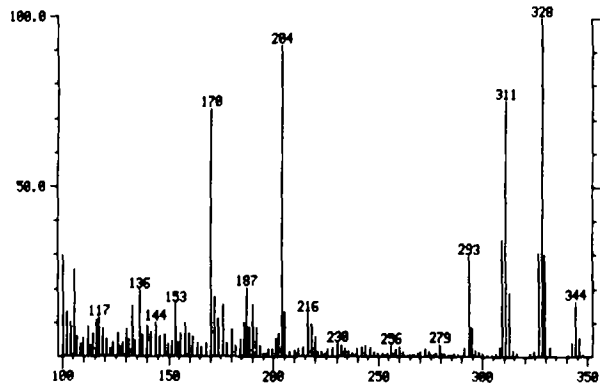

FIG. 9. Ammonia CI-MS of methyl 9-hydroperoxy-*trans*-10,-*cis*-12-octadecadienoate by direct insertion probe.

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Characteristics and Utilization of Dry Roasted Air-Classified Navy Bean Protein Fraction[1]

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ABSTRACT

Navy beans, *Phaseolus vulgaris*, were dry roasted in a particle-to-particle heat exchanger, dehulled by air aspiration, pin-milled and air-classified to yield a high protein fraction. Proximate analyses, nitrogen solubility indices and oligosaccharide contents of this high protein fraction as influenced by processing parameters which affected final product temperature were determined. Farinograms of wheat/bean protein fraction composite flours were run. A high-protein bean flour fraction was selected from these dry and roasted treatments and used in product development. Quality characteristics and consumer acceptability of high-protein prototype products were evaluated. Results of this research indicate that the dry roasting process influences the characteristics of the air-classified protein fraction. Flour color, nitrogen solubility and dough mixing properties were most greatly influenced by roasting time and temperature. Increased roasting resulted in increased browning and decreased nitrogen solubility and dough mixing stability. Wheat flour bread products, substituted with low levels of high-protein bean flour, were of high quality.

INTRODUCTION

The nutritional composition of dry navy beans is ideal for delivery of protein and minerals if properly prepared and positioned in the diet. Dry beans contain various metabolic inhibitors which must be inactivated or eliminated prior to consumption if maximum nutrient potential is to be derived. Increases in nutritional quality have been demonstrated by heating to destroy the heat labile antinutritional protease inhibitors and trypsin inhibitory compounds (1).

Lysine, which accounts for ca. 90% of the free amino groups in the form of epsilon-amino lysl residues, and methionine are highly reactive and limiting amino acids. The loss of epsilon amino groups of lysine occurs by condensation reaction with reducing sugars (2). These Maillard type browning reactions may occur in stored bean products and contribute to a loss of nutritive value and decline in sensory quality.

Heating of foods containing protein results in changes in water status, solubility of the protein and other changes in functionality of protein (3).

Chang and Satterlee (4) produced bean protein concentrates containing 72-81% protein by wet processing using water extraction techniques; Molina and Bressani (5) prepared protein isolates containing ca. 90% protein.

It has been observed, that raw legumes ground without pretreatment develop undesirable odors and flavors which persist after cooking. Lipoxidases have been held responsible for the appearance of off-flavors by catalyzing formation of hydroperoxides from unsaturated fatty acids (6). The highest lipoxidase activity experienced in pulses and oilseeds occurs in soybeans. However, treatment with dry heat for 6-8 min at 104-105 C completely inactivates this enzyme (7).

The objective of this research was to evaluate the effects of selected dry roasting treatments on the composition and functional properties of air-classified navy bean protein. A selected heat treated/bean protein was used in several wheat flour based products.

MATERIALS AND METHODS

Protein Fraction Preparation

Navy beans, *Phaseolus vulgaris*, were dry roasted in a particle-to-particle heat exchanger with control of bead temperature (240 C, 270 C), bean/bead ratio (1/10, 1/15), and roasting time (1 min, 2 min) (8). Roasted navy beans were dehulled

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