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False Positive Halogen Responses on HECD by Fatty Acids

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One of the cornerstones of an architectural approach adopted by this laboratory (Luke & Masumoto 1982) in screening vegetables for pesticides and industrial chemicals is the extensive use of the Hall electrolytic conductivity detector (HECD). Design improvements (Anderson & Hall 1980) have resulted in the analytical capability of detecting nanogram quantities of organonitrogen compounds and sub-nanogram amounts of organosulfur and organohalogen compounds. Routine use of such capabilities, however, is not without certain problems. Few interfering background peaks have been encountered due to the acetone-dichloromethane-petroleum ether partitioning clean-up procedure used (Luke et al. 1981). Since effluents from the gas chromatograph (GC) are normally ionized or reduced to produce an ionic species when dissolved in a suitable solvent, some compounds, if present in sufficient quantities can result in carbon dioxide production as a conducting species. Recently two strong responses on the HECD-X from bananas and eggplant extracts were not due to halogens. Further investigations by gas chromatography mass spectrometry (GCMS) revealed the compounds to be fatty acids.

This paper describes the identification of the encountered residues in eggplant to be a mixture of palmitic, oleic, stearic, linoleic and linolenic acids via chemical ionization (CI) GCMS.

MATERIALS AND METHODS

All chromatograms were obtained on a Tracor Model 560 gas chromatograph equipped with a Hall Model 700A electrolytic conductivity detector in the halogen mode: operating conditions; 120 cms x 2 mm i.d. glass column packed with 3% SP2100 on 80/100 mesh Chromosorb W.H.P.; carrier gas 10 mL/min H_2; column inlet 200°C; column temperature 180°C; detector operating conditions were as previously described (Luke et al. 1981).

100 g portions of ground egg plant were extracted (Luke et al. 1981) and concentrated to 4 mL and 7 mG (sample equivalents) injected onto the GC. Derivatization of the sample was performed by evaporating to an oily residue without heat under a stream of nitrogen followed by addition of 0.5 mL Methyl 8^{R} (DMF dimethyl acetal in pyridine) at room temperature. The reaction mixture was then injected directly onto the Silar 10C column attached to the GCMS.

All spectra were obtained on a Finnigan 3300 quadrupole mass spectrometer equipped with a CI source and INCOS data system; operating conditions: 45 cm x 2 mm i.d. glass coluumn packed with 2% DEGS on 80/100 mesh Chromosorb W for fatty acids and 150 cm x 2 mm i.d. glass column packed with 3% Silar 10C on 100/120 mesh Gas Chrom Q for fatty acid esters; carrier gas and reagent gas for CI: 25 mL methane/min, column inlet 250^{O}C, column temperatures 170^{O}C, isothermal; electron energy 150 eV.

RESULTS AND DISCUSSION

The initial findings of the two major responses by HECD-X (Figure 1) seemed to indicate the presence of at least two chlorinated compounds. Due to the broad peak shapes on SP2100 these molecules were thought to be polar entities. Such strong responses became of immediate concern. Experienced intuition led to the preliminary conclusion that such responses were probably not chlorinated, because of base-line drift and non-linearity of response on repeated injections. In the HECD system, the conducting species normally formed in the oxidative modes are carbon dioxide, the sulfur oxides and the halogen halides. Responses due to CO_2 are minimized by the use of an non-aqueous solvent such as n-propanol. However, there does exist the real possibility that some molecules present in substantial amounts (above 1000 ng per injection) can give a response on the HECD particularly if they contain oxygen. With this background information, it was imperative to resolve the identity of these observed responses.

Under GCMS conditions using a 2% DEGS column with methane as carrier and reagent gas for chemical ionization (Figure 2A), the identity of the eluting compounds were found to be fatty acids (Figure 3). Comparison with reference standards substantiated these conclusions. To assist the reader with the elution order of these fatty acids, mass chromatograms (Figure 2B) were constructed using the ion values unique to each fatty acid. It was interesting to observe that under methane CI conditions palmitic acid (Figure 3A) did not fragment extensively to hydrocarbon entities in the highly characteristic pattern observed for its homologs ($[C_nH_{2n+1} - 1]^+$ for the saturated and $[C_nH_{2n} - 1]^+$ for unsaturated, etc). While such characteristic patterns

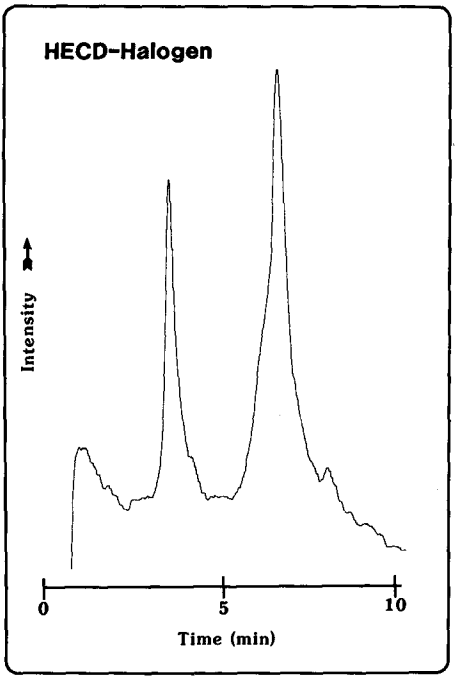

Figure 1. Gas chromatogram of eggplant extract obtained using HECD-X.

were clearly observerable for stearic (Figure 3B), linoleic (Figure 3C) and linolenic (Figure 3D) acids, the observance of an $[M - 1]^+$ ion at m/z 279 rather than the $[M + 1]^+$ ion for linoleic is unusual. With the presence of carboxylic groups, protonation at the carbonyl oxygen atom would be predicted. The experimental evidence that proton abstraction took place (admittedly to a minor extent) would strongly favor initial ionization at a site other than the carbonyl. Since the remainder of the molecule is hydrocarbon in nature, then protonation abstraction would be expected at the double bond site. The apparent absence of oleic acid or the lack of evidence to support its presence warranted that the extract be esterified and re-examined. Such additional experimental work would also serve to confirm the initial findings. On this occasion, however, a different stationary phase was employed (Silar 10C) to ensure an improved separation of the methyl esters of these fatty acids (Figure 4). Based on the full mass spectral scans (m/z 100-500) (Figure 4A) obtained from the reconstructed ion current (RIC), mass chromatograms (Figure 4B) were plotted to indicate the presence of palmitic, stearic, oleic, linoleic and linolenic acids as their methyl esters. In this case, the presence of methyl oleate was demonstrated. As a result of methylation, an unidentified peak was also observed (Figure 4A) and was concluded not to be of fatty acid origin.

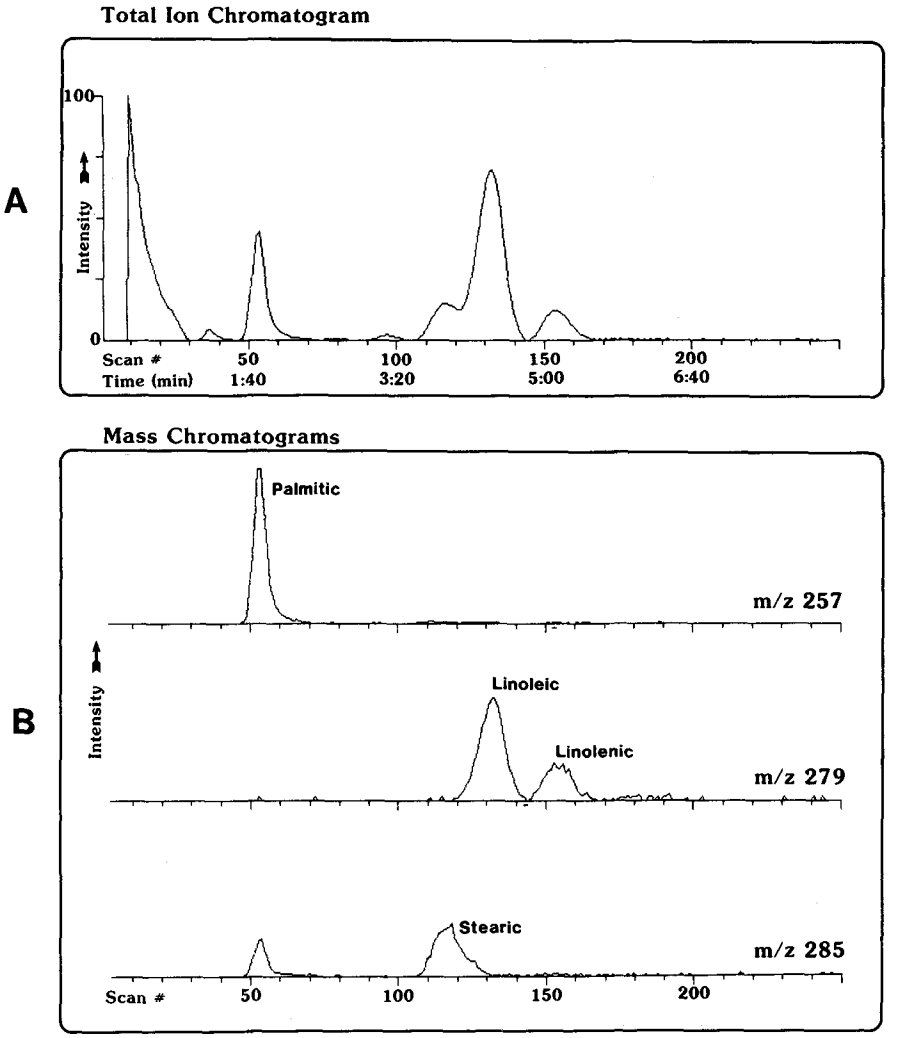

Figure 2. GCMS of eggplant extract: (A) Total ion chromatogram, and (B) mass chromatograms to distinguish constituent fatty acids.

A closer examination of the spectra of these methyl esters of fatty acids (Figure 5) was interesting. In the case of palmitic (Figure 5A) and stearic (Figure 5B),] both saturated entities, little or no fragmentation definitive of the presence of the long chain hydrocarbon moiety was evident. The base peak was the protonated molecular ions at m/z 271 and 299 respectively. However, the next most abundant ions represented proton abstraction, i.e. m/z 269 and 297 respectively. It would appear, that under CI a long chain methyl ester can behave as a proton donor or acceptor depending on the site of initial attack by CH_5^+.

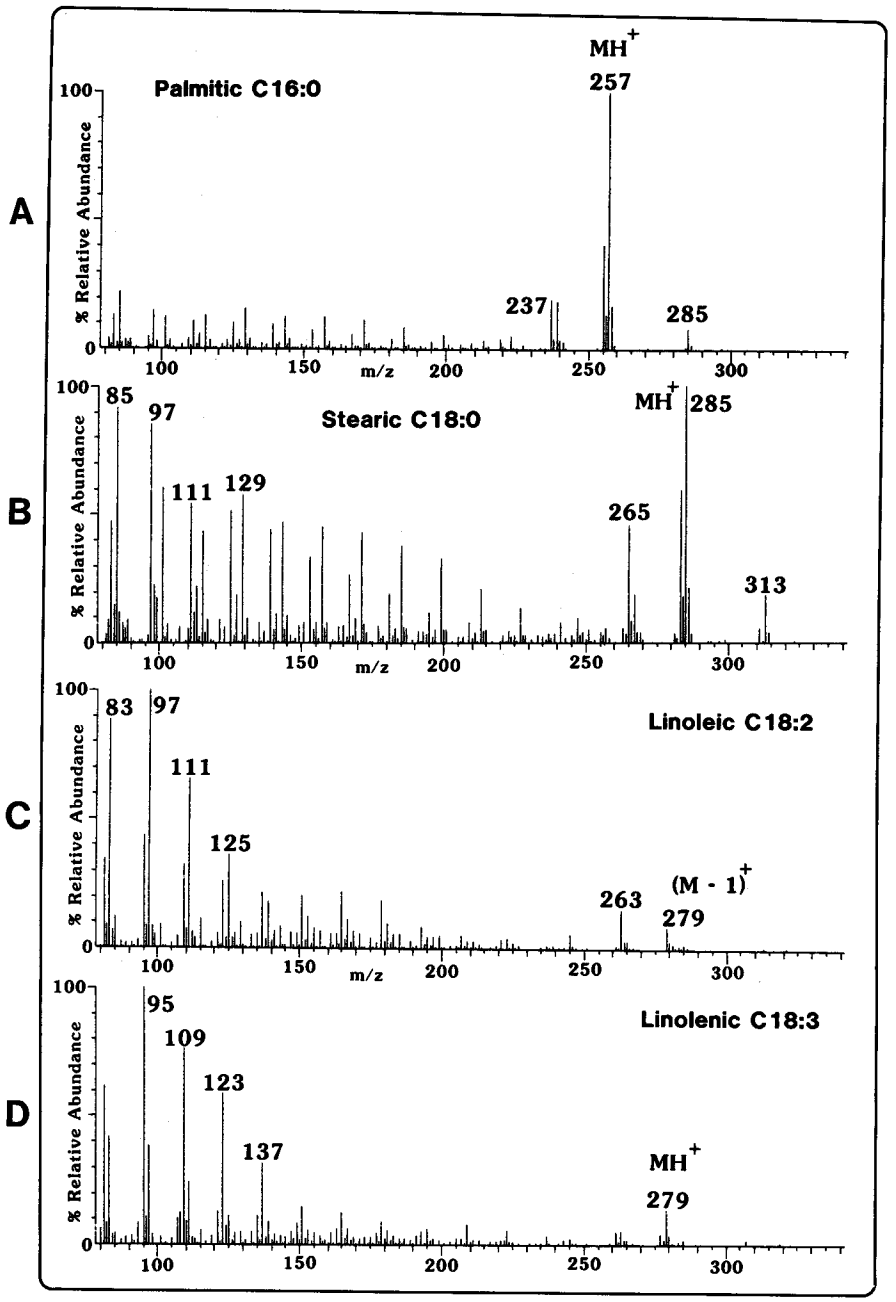

Figure 3. Full mass spectral scans obtained from peaks observed in eggplant extract.

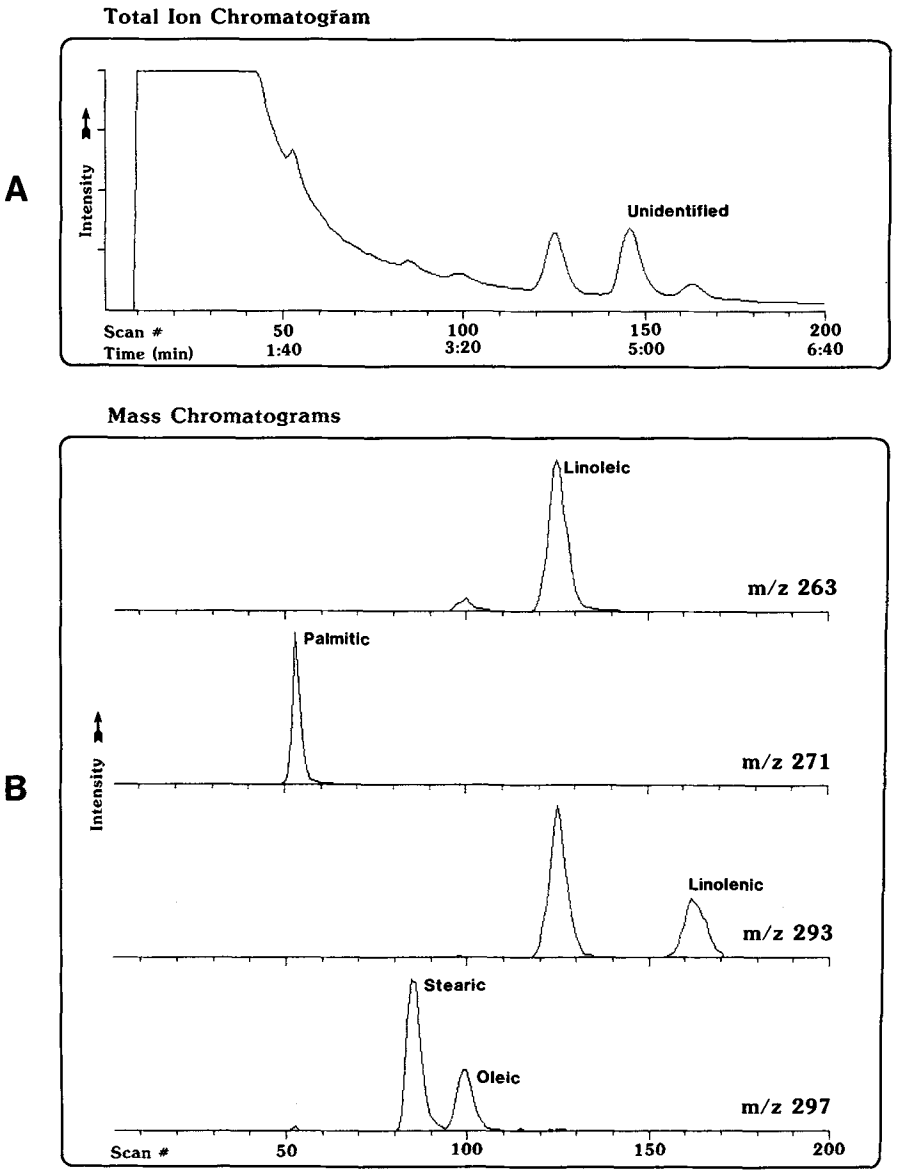

Figure 4. GCMS of methylated extract of eggplant: (A) total ion chromatogram, and (B) mass chromatograms to distinguish the constituent methyl esters.

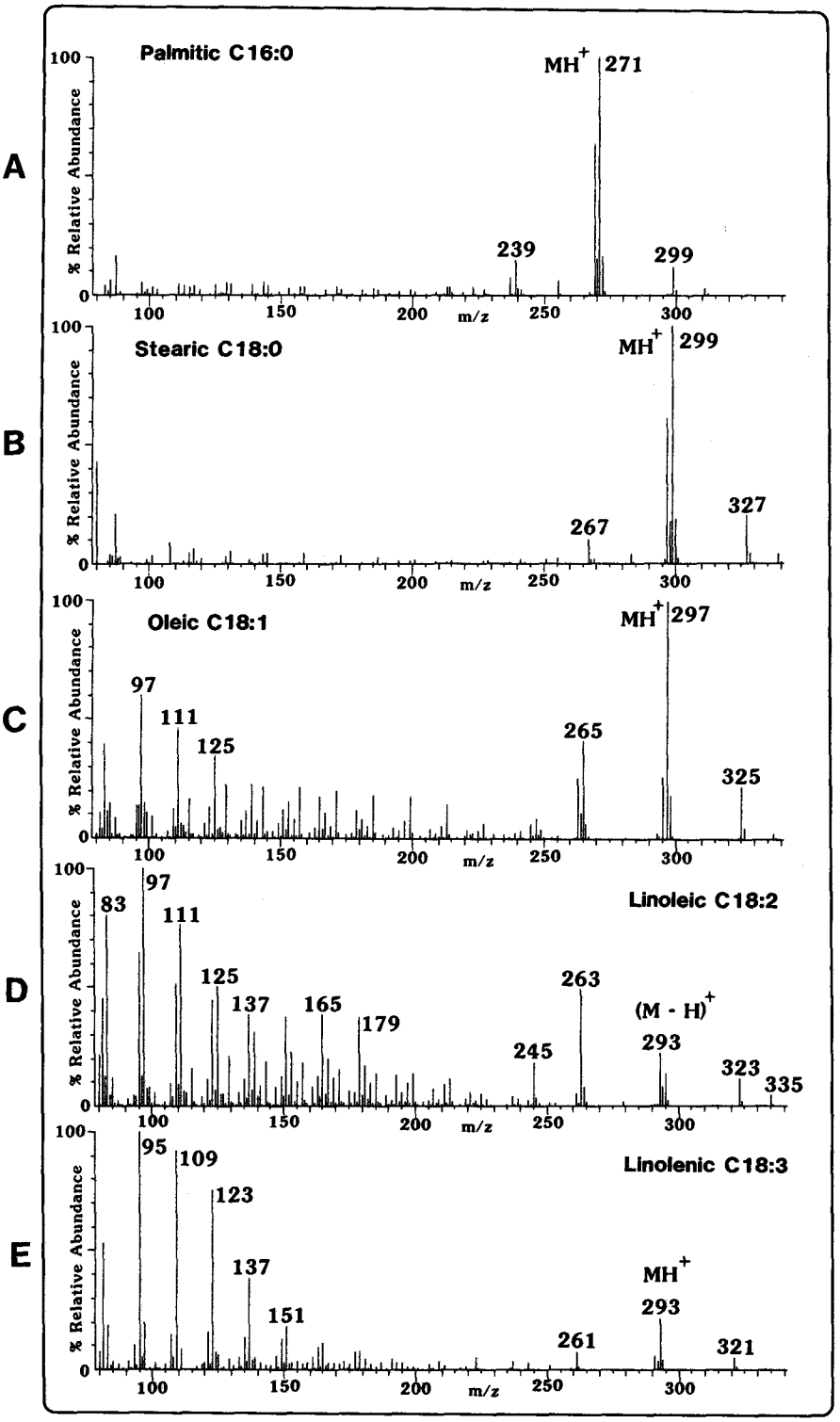
A
Palmitic C16:0
MH+ 271
239
299
% Relative Abundance
100
0
100
150
200
m/z
250
300
B
Stearic C18:0
MH+ 299
267
327
C
Oleic C18:1
MH+ 297
97
111
125
265
325
D
Linoleic C18:2
97
83
111
125
137
165
179
263
(M - H)+
245
293
323
335
E
Linolenic C18:3
95
109
123
137
151
MH+
293
261
321

Figure 5. (Appearing on previous page). Total mass spectral data obtained from peaks in methylated eggplant extract.

Turning now to the unsaturated methyl esters, the highly characteristic pattern of hydrocarbon fragmentation was clearly evident. Once again, however, linolenic (Figure 5D) has displayed the preferential tendency to proton abstract (i.e. m/z 293 representing $[M - 1]^+$).

Having established that the original responses by HECD were a mixture of fatty acids, an attempt was made to rationalize the observed experimental findings. While such fatty acids are capable of producing CO_2 via oxidation, they can also produce H_2O. Perhaps it is the combination of these two in gross amounts that were responsible for the observed responses. To gain an estimate of the quantity of fatty acids present in the extract, an injection of stearic acid necessary to produce such a response contained 2000 ng. It now became clear that the eggplant extract did contain large amounts of fatty acids which had navigated our extraction and clean-up procedures and been injected on column. While such occurrences are rare, the presence of fatty acids in eggplant deserves exposure in the literature. Residue analysts working with the HECD should now be aware of the prevalence of such residues in both eggplant and bananas.

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