Electrospray Ionization MS of High M.W. TAG Oligomers

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ABSTRACT: Reported are the on-line LC/electrospray ionization MS of large, high M.W. oligomers formed from heated triolein, a TAG used as a model for dietary oils. Triolein, the major component of olive oil, canola oil, and other dietary oils, was heated at frying temperature, and the TAG oxidation products were separated using RP-HPLC coupled to an ion trap mass spectrometer *via* an electrospray ionization interface. Ammonium formate was added as a sheath liquid to promote ammonium adduct formation. Masses corresponding to ammonium adducts of intact carbon-linked dimers (*m/z* 1783–1787), trimers (*m/z* 2666–2672), and tetramers (*m/z* 3547–3557) of triolein, with and without additional sites of unsaturation, were observed. Also, dimers, trimers, and tetramers containing one, two, or three additional oxygens, also with and without additional sites of unsaturation, are reported. Based on the formation of some types of triolein dimers, we believe that tristearin might also form dimers, even though it has no readily oxidizable sites of unsaturation. Oxidized tristearin monomers, tristearin dimers, chain-addition products, and chain-shortened products are observed.

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The reactions of atmospheric oxygen with fatty acyl chains to form lipid oxidation products have been studied extensively. Many reviews, book chapters, and books dedicated to lipid oxidation products have been published. One excellent reference describing the various aspects of lipid oxidation at ambient temperature was edited by Chan (1). Frankel has published extensively in the area of lipid oxidation, including thorough review articles (2,3). Studies on the oxidation of model FAME such as methyl oleate and methyl linoleate have long shown that oxidation of lipids initially produces hydroperoxides as the primary oxidation products, and these react further to produce a wide array of secondary products. Early work on the mechanisms and products of oxidation was performed on FA and their methyl esters because these were amenable to GC and GC/MS. Analysis of the FAME of oxidation products was also conducted using direct-exposure probe MS, NMR spectrometry, and others (such as IR spectrometry).

At least three broad categories of lipid oxidation products are produced by oxidation of FAME: (i) small volatile oxidation products, (ii) FA fragments, and (iii) dimers, trimers, and other oligomers. The small volatile molecules produced by the breakdown of hydroperoxides have been thoroughly discussed by Frankel (2), Grosch (4), and others. The small volatile oxidation products include alkanes, alkenes, alcohols, aldehydes, ketones, acids, and others. When a small volatile fragment breaks off from the fatty chain, a FA fragment is left behind as a "core aldehyde" or similar residue. The third type of oxidation product, oligomers, are formed when two fatty chains become linked at a site of oxidation. Several classes of dimers of methyl linoleate hydroperoxides were discussed by Miyashita and co-workers (5–7). These were identified by gel permeation chromatography followed by solid-phase extraction and then field desorption MS of derivatized and underivatized products.

The volatile and nonvolatile oxidation products formed from intact TAG also have been studied extensively. The small volatile oxidation products that break off a TAG are amenable to GC/MS, just like the volatiles produced from simple FA. The large intact TAG oxidation products (TAGOX) are not volatile, and so LC has been used for their separation. Mono-, bis-, and tris-hydroperoxides of TAG have been studied by Frankel, Neff, Miyashita, and co-workers (8–10). Hydroperoxides were saponified, transmethylated, and derivatized to their trimethylsilyl ethers (OTMS) and then analyzed using GC and GC/MS. The intact TAG hydroperoxides were analyzed using HPLC with refractive index (RI) or UV detection. The positional distribution of the hydroperoxide groups was determined by lipase hydrolysis. None of these methods, though, constituted the direct on-line HPLC/MS analysis of TAGOX.

High M.W. (HMW) TAGOX are produced by the joining together of intact monomeric TAGOX in a manner analogous to the FA oligomers mentioned above. Since these are large, nonvolatile molecules, they are not amenable to GC or GC/MS analysis. Hopia (11–13) and Marquez-Ruiz (14,15) and co-workers and others have employed size exclusion chromatography (SEC) for their separation and identification. Oligomeric components were detected using RI or ELSD. Although these 2-D detectors provide a quantitative estimate of the total amount of oligomers formed, they do not provide any information about the specific structures of the numerous possible classes of HMW TAGOX possible within a relatively narrow M.W. range (the basis of separation by SEC).

Neff and Byrdwell (16) reported the use of HPLC with atmospheric pressure chemical ionization (APCI) MS of monomeric TAGOX produced from the autoxidation of model TAG, and then from autoxidation of normal and genetically modified canola varieties (17). They then reported the products of heated oxidation at frying temperatures of triolein

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