Analysis of Plant Sterol Oxides by Liquid Chromatography/mass Spectrometry

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Analysis of plant sterol oxidation products has mainly been performed with gas chromatographic (GC) techniques. Recently liquid chromatographic (LC) techniques have become more popular. The major advantage of LC, compared to GC, is operating at room temperature and, therefore, the detection of thermolabile primary oxidation products, hydroperoxides, is possible without time-consuming derivatization. However, commonly applied LC detection methods require good separation of individual oxides, and this restricts the number of oxides that can be analysed in the same run. Recently, LC with mass spectrometric (MS) detection has become more common for the analysis of cholesterol oxidation products. MS detection allows identification and quantification of partially or totally co-eluting analytes. The aim of this study was to develop a LC-MS method for identification and quantification of diverse group of plant sterol oxides formed during oxidation.

The plant sterol oxides formed during thermo-oxidation at 180°C were separated based on their functional groups with normal-phase LC using heptane-isopropanol gradient elution. The MS detection was carried out with a quadropole ion-trap mass spectrometer using atmospheric pressure chemical ionization in positive ion scanning mode. For identification of oxides the stepwise fragmentation (MS^2 and MS^3) was used. Compounds with a conjugated diene structure had the protonated molecule ion $[M+H]^+$ as the base peak. In general, fragments indicating a loss of one $[M-H_2O+H]^+$ or two $[M-2H_2O+H]^+$ water molecules were observed for secondary oxidation products. In addition to loss of water molecules, the fragments representing loss of hydrogen peroxide $[M-H_2O_2+H]^+$, or hydrogen peroxide and water $[M-H_2O_2-H_2O+H]+$ were present in the mass spectra of plant sterol hydroperoxides. Sitosterol, campesterol, stigmasterol and brassicasterol oxides had similar fragmentation behaviour when compared to each other, only relative ion abundances were slightly different. The results showed that good chromatographic separation based on functional groups of plant sterol oxides coupled with specificity of MS detection made it possible to quantify as many as 34 major oxidation products of plant sterols formed during thermo-oxidation.