Detection of dimeric plant sterols formed during thermal oxidation by RP-HPLC-MS

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Plant sterols are thought to have cholesterol lowering abilities and are, therefore, added to foods as a functional food ingredient. The main mechanism of decomposition of plant sterols is oxidation. A lot is known about autoxidation reactions of plant sterol at temperatures at or below 100°C, but much less is known about the reactions taking place at higher temperatures. Recently, the monomeric thermal oxidation products have been indentified. However, a difference in mass balance between the initial amount of plant sterols and the amount of monomeric products found back after oxidation, shows that a large part of the products formed is still unidentified (Soupas *et al.*, 2005, Eur. J. Lipid Sci. Technol., 107, 107-118). This difference in mass balance might be partially explained by the formation of dimeric and polymeric oxidation products. This study focused on the analysis of dimeric thermal oxidation products of plant sterols.

A commercially available plant sterol, stigmasterol, was heated and oxidized for several hours at 180°C. Three fractions with different polarities were collected by solid phase extraction. Of each fraction, monomers, dimers and polymers were separated by size exclusion chromatography. The most polar dimeric fraction was used for method development. Dimeric thermal oxidation products were separated by HPLC both on normal phase and on reversed phase columns under several elution conditions. Best separation was obtained by reversed phase HPLC with a linear gradient from methanol to isopropanol.

Furthermore, an LC-MS strategy for the assignment of dimers was developed. Two ionization methods, APCI-MS and Ag^+-CIS-MS, were needed to assign most of the peaks. Tentative assignments of the peaks suggested that each ionization method led to the detection of a structurally different population of dimers.

In conclusion, this study showed that various dimeric oxidation products were formed during thermal oxidation of plant sterols. Dimers could be assigned tentatively, based on the developed RP-HPLC-MS method.