APPLICATION OF ELECTROSPRAY MASS SPECTROMETRY TO THE ANALYSIS OF LIPIDS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN FOOD TECHNOLOGY AT MASSEY UNIVERSITY

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Abstract

This work investigated the application of electrospray mass spectrometry (MS) to the elucidation of lipid structure, especially related to milkfat. This was the first time that the instrument in our laboratory had been used for this purpose.

Standard samples of triacylglycerols (TAGs) were prepared in 1,2 dichloroethane: acetonitrile:formic acid (63:35:2) at approximately 0.005 mg/mL and were then used to optimise and quantify the ion signal (response) generated by the Perkin Elmer Sciex API300 electrospray mass spectrometer. Both MS and tandem MS experiments were performed. In the spectra, the TAG molecule was seen as an ammonium ion adduct $(M+NH_4)^+$. It was found that the relative responses of diacylglycerol (DAG) ions, formed during front end fragmentation of the $(M+NH_4)^+$ ion, depended on the position of the 'lost' fatty acid on the glycerol backbone and its carbon number, with the former rather than the latter being the more critical. This information enabled the fatty acid esterified to the sn2 carbon of the glycerol backbone to be identified, and also demonstrated that it was possible to identify each of the fatty acids in a TAG molecule accurately by molecular and DAG ion identification. MS/MS experiments were performed on DAG ions, rather than parent ions, to identify and measure the response of acylium ions generated during collision-assisted dissociation (CAD). In contrast to the response of the DAG ions above, it was found that the response of these acylium ions was dependent on their carbon number and degree of saturation rather than the position that the fatty acid had held on the glycerol backbone. Optimal voltage settings for analysis of TAGs by infusion MS were obtained, which gave good quality spectra and ample amounts of molecular and DAG ions. With this information, a novel liquid chromatography-mass spectrometry (LC-MS) method, which was able to characterise the TAGs in a number of complex lipid samples, was developed. The method was used to elucidate differences in the TAG structure of different bovine milkfats and also differences in fat from milk of various mammalian species.

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Nomenclature

Common Names of Fatty Acids and their Carbon Numbers

Carbon number	*Systematic name*	*Common name*	*n*	*Molecular weight*
4:0(B)	butanoic	butyric		88.11
6:0	hexanoic	caproic		116.16
8:0	octanoic	caprylic		144.21
10:0	decanoic	capric		172.27
12:0	dodecanoic	lauric		200.32
14:0	tetradecanoic	myristic		228.38
16:0(P)	hexadecanoic	palmitic		256.43
16:1	hexadec-9-enoic	palmitoleic	7	254.41
17:0	heptadecanoic	margaric		270.46
18:0(S)	octadecanoic	stearic		284.48
18:1	octadec-6-enoic	petroselenic		282.47
18:1(O)	octadec-9-enoic (*cis*)	oleic	9	282.47
18:1(E)	octadec-9-enoic (*trans*)	elaidic	9	282.47
18:1	octadec-11-enoic (*trans*)	vaccenic	7	282.47
18:1	12-hydroxy-octadec-9-enoic	ricinoleic	9	298.47
18:2	octadeca-9,12-dienoic	linoleic	6	280.45
18:2c	octadeca-9,11-dienoic	conjugated linoleic		280.45
18:3c	octadeca-9,11,13-trienoic	elaeostearic		278.44
18:3	octadeca-9,12,15-trienoic	linolenic (alpha)	3	278.44
18:3	octadeca-6,9,12-trienoic	gamma linolenic	6	278.44
18:5	octadecapentaeonic			274.4
20:0	eicosanoic	arachidic		312.54
20:1	eicos-9-enoic	gadoleic		310.52
20:4	eicosa-5,8,11,14-tetraenoic	arachidonic	6	304.47
20:5	eicosa-5,8,11,14,17-pentaenoic	EPA	3	302.46
22:1	docos-11-enoic	cetoleic		338.58
22:1	docos-13-enoic	erucic	9	338.58
22:4	docosa-7,10,13,16-tetraenoic	adrenic	6	332.53
22:5	docosa-7,10,13,16,19-pentaenoic	clupadonic	3	330.51
22:6	docosa-4,7,10,13,16,19-hexaenioc	DHA	3	328.50
23:0	docosanoic			354.6
24:0	tetracosanoic	lignoceric		368.65
25:1	pentacosenoic			380.70

The triacylglycerols are abbreviated in the text as groups of individual fatty acids *e.g.* 16:0, 18:1, 18:0. Common triacylglycerols have been further abbreviated using the singly letter designations shown in the table above, *e.g.* POS for 1-palmitoyl-2-oleoyl-3-stearoylglycerol.

Diglycerols follow a similar pattern of abbreviation.

Unless stated no specific regioisomers are implied by the order of the nomenclature.