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Analytical Methods

Identification and quantification of phosphatidylinositol in infant formulas by liquid chromatography–mass spectrometry

Zhiqian Liu [a], Ben Cocks [a,b], Andy Patel [c], Alex Oglobline [c], Graeme Richardson [c], Simone Rochfort [a,b,*]

[a] Biosciences Research, AgriBio, 5 Ring Road, La Trobe University, Bundoora, Victoria, Australia
[b] School of Applied Systems Biology, 5 Ring Road, Bundoora, La Trobe University, Australia
[c] DTS Food Laboratories, Flemington, Australia

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ABSTRACT

Using LC-LTQ-Orbitrap MS we were able to identify 10 major phosphatidylinositol (PI) species present in 32 infant formulas (IF) collected from Australia, Europe and the USA. Based on the species fingerprint, the 32 formulas can be classified into several distinct groups by PCA analysis; this grouping pattern reflects origin and the label information of the formulas. The total content of all PI species was determined by LC-Triple Quadrupole MS in negative MRM mode using external standard calibration. The content of PI showed large variation between formulas and was very high in certain cases, which is believed to be related to the use of soybean lecithin in these products. Our study indicates that the content and speciation of PIs have significant contribution to the total amount of inositol in all 32 products surveyed; this contribution may be important for the fine nutritional profile and biological functions of IF products.

1. Introduction

Myo-inositol is one of the most abundant sugars in nature. Grains, beans, nuts and fresh fruits are good sources of inositol (Clements & Darnell, 1980). It is present both in its free form and as a component of phosphatidylinositol (PI) in cell membranes. Inositol and its derivative inositol hexaphosphate (IP6) play an important role in various biological functions, including protection against cancer, cell signalling, formation of the neural system and so on (Berridge & Irvine, 1989; Greene & Copp, 1997; Vucenik & Shamsuddin, 2006). Relatively high concentrations of inositol are found in human breast milk (Brown, Cheung, Harwood, & Battaglia, 2009), suggesting that exogenous inositol is required for postnatal development of newborns. To meet the requirement of formula-fed infants, inositol is generally added to infant formulas, with a minimum concentration of inositol in infant formulas required in certain countries (U.S. Food and Drug Administration, 2014).

The amount of free inositol in biological samples can be determined by enzymatic assay (Ashizawa, Yoshisa, & Aotsuka, 2000), gas chromatography (March, Forteza, & Grases, 1996), high-performance liquid chromatography (Lauro, Graven, & DeRubertis, 1989), and liquid chromatography–mass spectrometry (Perello, Isern, Costa-Bauza, & Grases, 2004). With regards to free and total inositol quantification in infant formulas, high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been the most widely employed technique (Chen, De Borba, & Rohrer, 2014; Schimpf, Thompson, & Baugh, 2012; Tagliaferri, Bonetti, & Blake, 2000), although the use of liquid chromatography–mass spectrometry (LC–MS) has also been reported (Flores, Moreno, Frenich, & Vidal, 2011).

Numerous reports are available on the quantification of PI and other polar lipids in milk samples. Normal phase liquid chromatography separation combined with evaporative light scattering detector (LC–ELSD) was the prevalent technique used for polar lipid quantification in milk in earlier studies (Avalli & Contarini, 2005; Rombaut, Camp, & Dewettinck, 2005). Although simple to use, LC–ELSD has clear limitations in that it can only determine the total content of PI but cannot provide any information regarding the species composition within the class. PI species composition in cow's milk has been characterised in a number of recent studies using LC–MS (Donato et al., 2011; Liu, Moate, Cocks, & Rochfort, 2015; Russo et al., 2013; Trenerry et al., 2013), but results obtained with raw milk cannot be extrapolated to infant formulas, because other PI-containing ingredient, such as soya lecithin, is often added to infant formulas (Le Grandois et al., 2009). By contrast, information on PI quantification in infant formulas is scarce; a single report was found that surveyed the level of five classes of

* Corresponding author at: Biosciences Research, AgriBio, 5 Ring Road, La Trobe University, Bundoora, Victoria, Australia.
E-mail address: simone.rochfort@ecodev.vic.gov.au (S. Rochfort).

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Table 1
PI species and isomers identified in IF and their fatty acid composition.

Code	$[M-H]^-$	Formula	Fatty acid composition[a]	
			Major component	Minor component
PI-1	831.5024	$C_{43}H_{77}PO_{13}$	C16:0-C18:3	
PI-2	833.5180	$C_{43}H_{79}PO_{13}$	C16:0-C18:2	
PI-3	835.5337	$C_{43}H_{81}PO_{13}$	C16:0-C18:1	
PI-4	857.5180	$C_{45}H_{79}PO_{13}$	C18:2-C18:2	
PI-5	859.5337	$C_{45}H_{81}PO_{13}$	a: C18:2-C18:1	b: C18:3-C18:0
PI-6	861.5493	$C_{45}H_{83}PO_{13}$	a: C18:2-C18:0	b: C18:1-C18:1
PI-7	863.5650	$C_{45}H_{85}PO_{13}$	C18:1-C18:0	
PI-8	883.5337	$C_{47}H_{81}PO_{13}$	a: C18:1-C20:4 b: C18:0-C20:5	c: C18:1-C20:4[b]
PI-9	885.5493	$C_{47}H_{83}PO_{13}$	a: C18:0-C20:4	b: C18:0-C20:4[b] c: C18:1-C20:3 d: C18:1-C20:3[b]
PI-10	887.5650	$C_{47}H_{85}PO_{13}$	a: C18:0-C20:3	b: C18:0-C20:3[b]

[a] The position (*sn*-1 or *sn*-2) of the two fatty acids are not defined.
[b] Positional isomers.

phospholipids in a small number of formula samples (Fong, Ma, & Norris, 2013). To our knowledge, detailed characterisation of PI species composition in infant formulas is lacking.

Although free inositol content in infant formulas is generally shown on the label, information on PI content and composition is never given. As PI can not only generate free inositol upon hydrolysis in human body, but also have additional health beneficial effects together with other phospholipid classes (for a review see Kullenberg, Taylor, Schneider, & Massing, 2012), a comprehensive characterisation of PI added intentionally or unintentionally in infant formulas is of great importance for accurately monitoring the ratio of free *versus* bound inositol in formula products. In addition, the absorption and metabolism of PI in humans may also be related to their acyl structure and whether the PI structure differs across different products remains totally unknown. The objectives of this work were to characterise the molecular species of PI present in different infant formulas and to survey the PI content in a large number of formula products. It is hoped that a thorough characterisation of infant formula PI can lead to a more rational use of inositol in improving infant nutrition.

2. Materials and methods

2.1. Infant formula samples

Thirty-two infant formula samples collected from Australia, Europe and the USA were tested in this work. A full-cream milk sample obtained from the local market was also included for comparison. Detailed information on these formula samples is given in Table 2.

2.2. Chemicals

PI (⩾99% purity from soybean) and myo-inositol (⩾99% purity) standards were purchased from Sigma–Aldrich. Solvents used for lipid extraction and mobile phase preparation were of chromatographic grade and were from Merck (methanol and acetonitrile), Sigma–Aldrich (chloroform and isopropanol) and Fisher Scientific (acetonitrile containing 0.1% formic acid). Ammonium formate, used as mobile phase additive, was of analytical grade (Sigma–Aldrich).

Table 2
PI and free myo-inositol contents of 32 infant formula samples.

Sample	Origin	Brand	Targeted age group	Myo-inositol (mg/100 g)[a]	PI (mg/100 g)[a]
#1	Australia	A	6–12 months	43.9 ± 1.3	119.7 ± 3.5
#2	Australia	A	6–12 months	42.6 ± 1.7	116.7 ± 2.6
#3	Australia	O	0–6 months	38.4 ± 0.6	37.7 ± 1.4
#4	Australia	O	0–6 months	39.7 ± 1.1	38.2 ± 1.6
#5	Australia	O	0–6 months	38.0 ± 1.5	38.3 ± 1.2
#6	Australia	S	0–12 months	32.6 ± 0.8	23.4 ± 1.5
#7	Australia	A	6–12 months	42.4 ± 1.2	115.7 ± 2.6
#8	Australia	O	0–6 months	38.6 ± 1.4	38.5 ± 1.1
#9	Australia	O	0–6 months	39.4 ± 2.1	37.3 ± 1.5
#10	Australia	O	0–6 months	38.5 ± 1.5	37.1 ± 2.2
#11	Australia	O	0–6 months	38.9 ± 0.9	38.6 ± 1.4
#12	Australia	O	0–6 months	39.2 ± 2.0	38.6 ± 1.9
#13	Australia	O	0–6 months	40.1 ± 1.2	37.7 ± 1.4
#14	Australia	O	0–6 months	39.7 ± 1.3	38.1 ± 1.2
#15	Australia	M	6–12 months	47.6 ± 1.9	47.6 ± 1.4
#16	Australia	M	12–36 months	47.2 ± 1.5	46.5 ± 1.0
#17	Australia	A	6–12 months	43.5 ± 2.0	114.7 ± 1.3
#18	Australia	O	0–6 months	38.5 ± 1.8	38.4 ± 1.1
#19	Australia	O	0–6 months	39.4 ± 2.5	37.9 ± 1.2
#20	Australia	O	0–6 months	39.9 ± 2.4	38.6 ± 1.0
#21	Australia	S	12–36 months	66.8 ± 3.5	29.0 ± 1.5
#22	Australia	O	0–6 months	40.6 ± 1.7	37.5 ± 1.1
#23	Australia	O	0–6 months	40.9 ± 1.9	38.6 ± 1.3
#24	Australia	O	0–6 months	41.4 ± 1.3	38.3 ± 1.4
#25	Australia	O	0–6 months	40.1 ± 0.9	38.2 ± 0.8
#26	Australia	O	0–6 months	40.8 ± 1.0	38.5 ± 1.5
#27	Australia	M	0–6 months	46.3 ± 1.2	46.6 ± 1.3
Euro-1	France	N	From birth	34.0 ± 0.4	74.5 ± 1.1
Euro-2	The Netherlands	N	From birth	62.5 ± 1.8	47.4 ± 1.5
Euro-3	Ireland	N	From birth	36.9 ± 1.4	46.1 ± 1.4
Euro-4	Germany	P	From birth	41.3 ± 1.6	11.5 ± 0.8
SRM1849	USA	NIST	Unknown	42.4 ± 1.8	13.3 ± 0.7
Milk	Australia	Procal	N/A	9.3 ± 0.1[b]	27.4 ± 0.8[b]

[a] Means of 3 replicates (±SD).
[b] Calculated based on 12.5% of total solid content.