Short chain lipids

Field of the Art

[001] The present invention relates to lipids comprising phospholipids and/or monoacylglycerides comprising esterified short chain fatty acids (SCFA), and their use in food and beverage products and feedstuffs.

Related Applications

[002] This application claims priority to Australian Provisional Patent Application No. 2023903391, entitled "Short chain lipids" and filed 24 October 2023; Australian Provisional Patent Application No. 2024900326, entitled "Short chain lipids" and filed 12 February 2024; Australian Provisional Patent Application No. 2024900423, entitled "Short chain lipids" and filed 21 February 2024, the content of each of which is incorporated by reference in its entirety.

Background

[003] As the global population surges towards a predicted 9.1 billion people by 2050, the demand for meat and dairy products for human nutrition is expected to continue to increase. However, meat and dairy production worldwide account for 70% of freshwater consumption, 38% of the total arable land use and contribute 19% of the world's greenhouse gas emissions. There is growing interest in finding alternative sources of protein and fat which have less of an environmental footprint. There is also a growing market worldwide for non-animal sources of high-quality protein and fat, for example from plant sources, which are seen as being more sustainable and environmentally friendly. Cultural and religious reasons have also contributed to growing markets for non-animal proteins. Sales of so-called plant milk, promoted as an alternative to dairy products, are predicted to surpass $34 billion globally by 2024. However, many current plant-based alternatives for meat and dairy products use fats made from blends of plant oils such as coconut, soy and palm oils which may give inadequate flavour and function. Fats and oils add flavour, lubricity and texture to foods and contribute to the feeling of satiety upon consumption, and therefore food and beverage products incorporating lipids from animal sources are often still preferred by consumers.

[004] Dairy products and alternatives can be obtained from mammalian milk, plant commodities and precision fermentation. While most dairy products consumed are made from mammalian milk, plant-based beverage consumption is on the rise around the world and represents the most consumed source of dairy alternatives (Sethi et al., 2016). Plant-based beverages can be produced from a variety of ingredients including cereal and pseudocereal (e.g., oat, rice, buckwheat, quinoa), nuts (e.g., almond, hazelnuts, cashew), legumes (e.g., soy) and seeds (e.g., hemp) (Reyes-Jurado et al, 2021). While the popularity of dairy alternatives is increasing worldwide, taste and functionality are major drawbacks to consumer acceptance and their application in food products (Aschemann-Witzel et al., 2021). There remains a need for animal free dairy ingredients that can improve the nutritional, sensory and functional properties of dairy alternatives.

[005] Bovine milk contains approximately 87% water, 4.6% lactose, 3.4% protein, 3.5-4.5% fat, 0.8% minerals and 0.1% vitamins (Mansson, 2008). Milk fat is a key component determining the

nutrition, texture, mouthfeel and flavour of dairy products. Milk fat is a complex biological material with unique structure and fatty acid composition. The lipids in bovine milk are mainly present in globules as an oil-in-water emulsion. The fat component consists of approximately 97-98% triacylglycerols (TAG), about 2% diacylglycerol (DAG), 0.5% cholesterol, 0.1% phospholipids (PL) and free fatty acids (FFA), although the composition of milk and its fat varies with the breed of cattle, type of feed, lactation stage and season. Milk fat is unusual in its fatty acid composition and functional properties, comprising as many as 400 different fatty acids, making it the most complex of all natural fats. Saturated fatty acids make up about 70% by weight of the fatty acid content of the TAG, including palmitic acid (C16:0) at about 30% or more by weight of the total fatty acids. Myristate (C14:0) and stearate (C18:0) are each present at more than 10% of the total fatty acid content, and short chain fatty acids (SCFA; C2:0, C4:0, C6:0 and C8:0) are together present at about 6-8% of the total fatty acid content. Monounsaturated fatty acids, primarily oleic acid (C18:1Δ9) at up to about 22% but also including low levels of C14:1 and C16:1 make up most of the non-saturated fatty acid content. Milkfat generally contains only low levels (<2%) of polyunsaturated fatty acids (PUFA), present as linoleic acid (C18:2) and linolenic acid (C18:3). The short chain fatty acids butyric acid (C4:0) and caproic acid (C6:0) present in milkfat are esterified almost exclusively at the sn-3 position of TAG molecules.

[006] Alternative dairy products often use plant-originated fats/oils such as canola oil, sunflower and soybean oil, which have different fatty acid composition and structure compared to mammalian milk. There remains a need for alternative, non-animal sources of lipids that have animal-fat like composition and functional properties, for human nutrition.

Summary of the disclosure

[007] In one embodiment, the present disclosure provides a lipid comprising phospholipids, wherein: a) the phospholipids comprise phospholipid esterified fatty acids, wherein the phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, optionally wherein: the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids; and/or the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC) and from about 15% to 35% phosphatidylethanolamine (PE); or b) the lipids comprise lipid esterified fatty acids, wherein the lipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, wherein at least 99% (w/w) of the lipid is phospholipids, and optionally wherein: the lipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids; and/or the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC), and from about 15% to 35% phosphatidylethanolamine (PE).

[007a] Disclosed herein is a lipid comprising phospholipids, wherein the phospholipids comprise phospholipid esterified fatty acids, wherein the phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, optionally wherein: the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol C4 fatty acids; and/or the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC) and from about 15% to 35% phosphatidylethanolamine (PE).

[008] In some embodiments, the phospholipids further comprise one, two, three or all of phosphatidylinositol (PI), phosphatidylserine (PI), phosphatidic acid (PA), and lysophospolipids. In some examples, the lysophospholipids are selected from lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) lysophosphatidylinositol (LPI), lysophosphatidylinositol (LPI) and lysphosphatidic acid (LPA). In particular examples, the phospholipids comprise one or more of the following: from about 0 to about 5% LPC; from about 5 to about 20% LPE; from about 1 to about 15% LPS; and from about 0 to about 10% PS; from about 0 to about 10% PI; and from about 0 to about 5% PA.

[009] In some embodiments, the lipid or phospholipid esterified fatty acids comprise from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 5.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; or from about from about 6 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids.

[010] In some embodiments, the lipid or phospholipid esterified fatty acids comprise from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; or from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids.

[011] In some embodiments, about 1.5% to 20% of the phospholipids are PC comprising esterified C4 fatty acids (C4-PC), and/or from about 1.5% to 20% of the phospholipids are PE comprising esterified C4 fatty acids (C4-PE). In particular examples, i) from about 1.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2.5% to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from

about 3 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3.5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; or from about 5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% of the phospholipids are C4-PC; and/or ii) from about 1.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2.5% to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3.5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; or from about 5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% of the phospholipids are C4-PE.

[012] In one embodiment, the lipid or phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C8 fatty acids. In another embodiment, the lipid or phospholipid esterified fatty acids comprise C16 and/or C18 fatty acids. In particular examples, the lipid or phospholipid esterified fatty acids comprise: from about 3 mol% to about 60 mol%; from about 5 mol% to about 55 mol%; from about 10 mol% to about 50 mol%; from about 10 mol% to about 40 mol%; from about 10 mol% to about 30 mol%; from about 10 mol% to about 60 mol%; from about 15 mol% to about 55 mol%; from about 15 mol% to about 50 mol%; from about 15 mol% to about 40 mol% ; from about 15 mol% to about 30 mol%; from about 15 mol% to about 25 mol%; from about 20 mol% to about 60 mol%; from about 20 mol% to 55 mol%; from about 20 mol% to 45 mol%; from about 3 mol% to about 30 mol%; from about 5 mol% to about 25 mol%; or from about 10 mol% to about 20 mol% esterified C16 fatty; and/or from 25 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol%; from about 30 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol%; from about 35 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol%; or from about 40 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol% C18 esterified fatty acids.

[013] In some embodiments, the C16 fatty acids comprise C16:0 fatty acids, and/or the C18 fatty acids comprise one or more or all of C18:0, C18:1, C18:2 and C18:3 fatty acids. In some examples, the lipid or phospholipid esterified fatty acids comprise any one or more of i)-v): i) from about 5 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 6 mol% to about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 7 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; from about 8 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; from about 9 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; or from about 10 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; ii) from about 1 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 1.5 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2.5 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19 or 20 mol%; or from about 3 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol% C18:0 esterified fatty acids; iii) from about 2 mol% to about 20, 25, 30, 35, 40, 45, 50, 55, 60 or 65 mol%; from about 5 mol% to about 20, 25, 30, 35, 40, 45, 50, 55, 60 or 65 mol%; from about 2 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 2 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 4 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%,; from about 5 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 6 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; or from about 7 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C18:1 esterified fatty acids; iv) from about 20 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%;from about 25 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; from about 30 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; from about 35 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; or from about 40 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol% C18:2 esterified fatty acids; and/or v) from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; or from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol% C18:3 esterified fatty acids.

[014] In a particular example, the lipid or phospholipid esterified fatty acids comprise from about 5 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 25 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 1 mol% to about 10 mol% C18:3 fatty acids.

[015] In some embodiments, the lipid comprises monoacylglycerides, wherein the monoacylglycerides comprise monoacylglyceride esterified fatty acids, and wherein the monoacylglyceride esterified fatty acids comprise C4 fatty acids, C6 fatty acids or a combination thereof. In one example, the monoacylglyceride esterified fatty acids comprise at least 1.5, 2, 3, 4 or 5 mol% C4 fatty acids, C6 fatty acids or a combination thereof.

[016] In particular embodiments, the lipid comprises at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% phospholipids (by weight), and/or no more than 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% monoacylglycerides. In further embodiments, the lipid comprises less than 15%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% triacylglycerides.

[017] In one embodiment, the present disclosure provides a lipid comprising monoacylglycerides, said monoacylglycerides comprising monoacylglyceride esterified fatty acids, wherein said monoacylglyceride esterified fatty acids comprise at least 2 mol% C4 fatty acids, C6 fatty acids or a combination thereof.

[018] In some embodiments, said monoacylglyceride esterified fatty acids comprise at least 2 mol% C4 fatty acids, for example at least 3 mol% C4 fatty acids. In some embodiments, said monoacylglyceride esterified fatty acids comprise at least 2 mol% C6 fatty acids, for example at

least 3 mol% C6 fatty acids. In some embodiments, the lipid further comprises phospholipids, said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 2 mol% short chain fatty acids. In some such embodiments, said phospholipid esterified fatty acids comprise at least 2 mol% C4 fatty acids, C6 fatty acids or a combination thereof, for example at least 2 mol% C4 fatty acids, for example at least 3 mol% C4 fatty acids. In some embodiments, said phospholipid esterified fatty acids comprise at least 2 mol% C6 fatty acids, for example at least 3 mol% of C6 fatty acids.

[019] In one embodiment, the present disclosure provides a composition comprising the lipid of the present disclosure, such as defined above and below. The composition may comprise one or more further components.

[020] In one embodiment, provided is a composition comprising a lipid comprising phospholipids, wherein the phospholipids comprise phospholipid esterified fatty acids, wherein the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids, and wherein: i) the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC) and from about 15% to 35% phosphatidylethanolamine (PE); ii) the phospholipid esterified fatty acids comprise from about 1.5 or 4 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 25 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 1 mol% to about 10 mol% C18:3 fatty acids; iii) the composition comprises from about 20% to about 70% phospholipid; and/or iV0 the composition comprises one or more of carbohydrates, protein and ash, optionally wherein the carbohydrates and/or protein are soy, sunflower, cottonseed or rapeseed carbohydrates and/or protein.

[020a] Disclosed herein is a composition comprising: the lipid as defined herein; or a lipid comprising phospholipids, wherein the phospholipids comprise phospholipid esterified fatty acids, wherein the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids, and wherein: i) the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC) and from about 15% to 35% phosphatidylethanolamine (PE); ii) the phospholipid esterified fatty acids comprise from about 2 or 4 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 25 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 1 mol% to about 10 mol% C18:3 fatty acids; iii) the composition comprises from about 20% to about 70% phospholipid; and/or iv) the composition comprises one or more of carbohydrates, protein and ash, optionally wherein the carbohydrates and/or protein are soy, sunflower, cottonseed or rapeseed carbohydrates and/or protein.

[021] In some embodiments, i) the total fat (by dry weight) of the composition is from about 30% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 35% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 40% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from

about 42% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 44% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 45% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 46% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 48% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; or from about 50% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; and/or ii) at least or about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% of the total fat is phospholipids.

[022] In some embodiments, the total fatty acids comprise about 1.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 % C4 fatty acids; from about 2 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 25, 30 or 35 % C4 fatty acids; from about 2.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 25, 30 or 35 % C4 fatty acids; from about 3 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 3.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 4 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 4.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; or from about 5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids. In some examples, the total fatty acids comprises about 70% to about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 75% to about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 80% to about 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 85% to about 90, 92, 93, 94 or 95 % long chain fatty acids; or from about 90% to about 93, 94, 95, 96, 97 or 98% long chain fatty acids.

[023] The compositions may comprise protein, such as from about 1 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 2 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 3 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 4 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); or from about 5 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight). In some examples, the compositions comprise carbohydrate, such as from about 5% to about 20, 25, 30, 35, 40 or 50 % carbohydrate (by weight); from about 6 % to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 7% to about 20, 25, 30, 35, 40% carbohydrate (by weight); from about 8% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 9% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 10% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); or from about 5% to about 10, 20, 25, 30, 35, 40, or 50% carbohydrate (by weight). In further examples, the compositions comprise ash.

[024] In some embodiments, the composition comprises the lipid, an aqueous component and a hydrophobic component. In some embodiments, the hydrophobic component comprises a non-polar lipid. In some embodiments, the non-polar lipid comprises triacylglycerides. In some embodiments,

the non-polar lipid is in the form of a vegetable oil. In some embodiments, the composition comprises lipid droplets. In some embodiments, the composition comprises the lipid as defined herein, wherein said phospholipids are present in the composition in an amount of at least 0.01% by weight.

[025] In some embodiments, the composition comprises one or more peptides or proteins, one or more carbohydrates and/or one or more bioactive agents. In some embodiments, said bioactive agents are selected from steroids, anti-oxidants, vitamins, minerals, fatty acids, amino acids, prebiotics, peptides, proteins, enzymes, drugs, antibodies, and hormones.

[026] In one embodiment, the present disclosure provides a delivery vehicle for delivering one or more components to a subject, the delivery vehicle comprising a composition of the invention described above and herein, wherein the composition comprises lipid droplets. In some embodiments, the delivery vehicle is for delivery of one or more bioactive agents. In some embodiments, said bioactive agents are selected from steroids, anti-oxidants, vitamins, minerals, fatty acids, amino acids, prebiotics, peptides, proteins, enzymes, drugs, antibodies, and hormones.
[026a] Disclosed herein is a delivery vehicle for delivering one or more components to a subject, for example for delivery of one or more bioactive agents, the delivery vehicle comprising the composition as defined herein wherein the composition comprises lipid droplets, optionally wherein said bioactive agents are selected from steroids, anti-oxidants, vitamins, minerals, fatty acids, amino acids, prebiotics, peptides, proteins, enzymes, drugs, antibodies, and hormones.

[027] In one embodiment, the present disclosure provides a food, beverage or feedstuff comprising the lipid according to the first or second, or the composition as defined herein.
[027a] Disclosed herein is a food, beverage or feedstuff comprising: a) a lipid comprising phospholipids, said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, optionally wherein: the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids; and/or the phospholipid esterified fatty acids comprise from about 1.5 or 4 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 20 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 11 mol% to about 15 mol% C18:3 fatty acids; or b) the lipid as defined herein; or c) the composition as defined herein.

[028] In one embodiment, provided is a food, beverage or feedstuff comprising a lipid comprising phospholipids, said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, optionally wherein: the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids; and/or the phospholipid esterified fatty acids comprise from about 1.5 or 4 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 20 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 11 mol% to about 15 mol% C18:3 fatty acids.

[029] In some embodiments, the food, beverage or feedstuff contains no components derived from an animal. In some embodiments, the food, beverage or feedstuff is a dairy product substitute. In some embodiments, the food, beverage or feedstuff is a dairy-free milk, dairy-free yoghurt, dairy-free cheese, dairy-free cream, dairy-free butter, dairy-free margarine, dairy-free chocolate or dairy-free ice cream.

[030] In some alternative embodiments, the food, beverage or feedstuff is a dairy product, for example a milk, yoghurt, butter, cheese, cream, ice cream or chocolate, for example a reduced fat milk.

[031] In some embodiments, the composition is present in the food, beverage or feedstuff in an amount of up to 5%, 4.5%,4%, 3.5%, 3%, 2.5%, 2%, 1.5%, 1%, 0.5%, 0.4%, 0.3%, 0.2% or 0.1% by weight, and/or the composition is present in the food, beverage or feedstuff in an amount of at least 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45% 0.5%, 0.55%, 0.6%, 0.65%, 0.7%, 0.75%, 0.8%, 0.85%, 0.9%, 0.95% or 1% by weight.

[032] In some embodiments, the lipid is present in the food, beverage or feedstuff in an amount of up to 2.5%, 2%, 1.5%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% or 0.01% by weight; and/or the lipid is present in the food, beverage or feedstuff in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45% or 0.5% by weight.

[033] In one embodiment, the present disclosure provides a method of producing a food, beverage or feedstuff, the method comprising mixing the lipid as defined herein or the composition as defined herein with at least one other consumable ingredient. In some embodiments, the food, beverage or feedstuff contains no components derived from an animal. In some embodiments, the food, beverage or feedstuff is a dairy product substitute (e.g. a dairy-free milk, dairy-free yoghurt, dairy-free cheese, dairy-free cream, dairy-free butter, dairy-free margarine, dairy-free chocolate or dairy-free ice cream). In some alternative embodiments, the food, beverage or feedstuff is a dairy product, for example a milk, yoghurt, butter, cheese, cream, ice cream or chocolate, for example a reduced fat milk.

[033a] Disclosed herein is a method of producing a food, beverage or feedstuff, the method comprising mixing the lipid as defined herein or the composition as defined herein with at least one other consumable ingredient.

[034] In one embodiment, the present disclosure provides use of the lipid as defined herein or the composition as defined herein to produce a food, beverage or feedstuff.

[034a] Disclosed herein is the use of the lipid as defined herein or the composition as defined herein to produce a food, beverage or feedstuff.

[035] In some embodiments, the food, beverage or feedstuff contains no components derived from an animal. In some embodiments, the food, beverage or feedstuff contains no components derived from an animal. In some embodiments, the food, beverage or feedstuff is a dairy product substitute (e.g. a dairy-free milk, dairy-free yoghurt, dairy-free cheese, dairy-free cream, dairy-free butter, dairy-free margarine, dairy-free chocolate or dairy-free ice cream). In some alternative

embodiments, the food, beverage or feedstuff is a dairy product, for example a milk, yoghurt, butter, cheese, cream, ice cream or chocolate, for example a reduced fat milk.

[036] In one embodiment, the present disclosure provides a process for preparing a lipid comprising phospholipids, comprising contacting a source of phospholipids, , said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, wherein: the method comprises contacting a source of phospholipids with: a) a triacylglyceride or diacylglyceride comprising esterified C4 and/or C6 fatty acids, butyric acid or caproic acid; and b) an enzyme suitable for effecting transesterification of the phospholipid with C4 and/or C6 fatty acids, for a time and under conditions sufficient to effect such transesterification, to thereby produce the lipid; and the source of phospholipids is a lecithin. In some embodiments, the lipids is as defined herein.

[036a] Disclosed herein is a process for preparing a lipid comprising phospholipids, said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, wherein: the process comprises contacting a source of phospholipids with: a) a triacylglyceride or a diacylglyceride comprising esterified C4 and/or C6 fatty acids, butyric acid or caproic acid, such as tributyrin; and b) an optionally immobilized enzyme suitable for effecting transesterification of the phospholipid with C4 and/or C6 fatty acids, for example a lipase, a phospholipase and/or a triacylglyceride lipase, for a time and under conditions sufficient to effect such transesterification, to thereby produce the lipid; and the source of phospholipids is a lecithin; optionally wherein the lipid is as defined herein; and optionally wherein the lecithin is a plant lecithin, such as a soy lecithin, sunflower lecithin, cottonseed lecithin or rapeseed lecithin, egg yolk lecithin, yeast lecithin or fungus lecithin; and optionally wherein the process further comprises purifying the lipid, optionally by acetone and/or ethanol precipitation or extraction; and optionally wherein the composition as defined herein is produced.

[037] In some embodiments, the enzyme is a lipase, a phospholipase or a triacylglyceride lipase. In some embodiments, the enzyme is immobilised. In some embodiments, the source of phospholipids is a lecithin, for example soy lecithin, sunflower lecithin, rapseed lecithin or egg yolk lecithin. In some embodiments, the triacylglyceride or diacylglyceride comprises esterified C4 fatty acids. In some embodiments, the triacylglyceride is tributyrin. In some examples, the process further comprises purifying the lipid, optionally by acetone and/or ethanol precipitation or extraction.

[038] In one embodiment, the present disclosure provides a lipid prepared by a process as defined herein, such as a lipid as defined herein.

[039] In one embodiment, the present disclosure provides a process of preparing a lipid, comprising contacting a triacylglyceride or diacylglyceride comprising esterified short chain fatty acids with an enzyme suitable for effecting conversion of the triacylglyceride or diacylglyceride to a monoacylglyceride comprising esterified short chain fatty acids, for a time and under conditions sufficient for effecting such conversion. In some embodiments, the enzyme is a lipase. In some embodiments, the enzyme is immobilised. In some embodiments, the triacylglyceride comprises esterified C4 fatty acids. In some embodiments, the triacylglyceride is tributyrin.

[040] In one embodiment, the present disclosure provides a lipid obtained by a process as defined herein.

[040a] Disclosed herein is a lipid prepared by the process as defined herein.

Brief Description of the Drawings

[041] **Figure 1** shows the C4 incorporation into phospholipid by transesterification using lipase and tributyrin. A) C4 incorporation with different ratios of lecithin:tributyrin and different lipase concentrations. B) C4 incorporation over time.

[042] **Figure 2** shows particle size distribution of water and oil emulsions with and without the addition of 0.5% (w/w) C4-Phospholipids. A) Particle size distribution of unhomogenized and homogenized full cream milk and water and oil emulsions with and without the addition of 0.5% (w/w) C4-Phospholipids (lipase batch 1) or soy lecithin. B. Particle size distribution of water and oil emulsions with and without the addition of 0.5% (w/w) C4-Phospholipids from lipase batch 1 or lypozyme batch 1.

[043] **Figure 3** shows the microstructure of C4-PL (lipase batch 1) emulsion showing the fat triacyl glycerol (TAG) core (A) and phospholipid (PL) rich membrane (B) in comparison with cow milk fat globule membrane (C) and a schematic diagram of a mammalian milk fat globule membrane (D). XO= Xanthine oxidase, ADPH = adipophilin, BTN = butyrophilin.

[044] **Figure 4** shows rheological properties of unhomogenized and homogenized full cream milk and water and oil emulsions with and without the addition of 0.5% (w/w) C4-Phospholipids (lipase batch 1) or soy lecithin.

[045] **Figure 5** shows rheological properties of skim milk and oil emulsions with and without the addition of 0.5% (w/w) C4-Phospholipids or soy lecithin.

[046] **Figure 6** shows the stability of a batter emulsion with and without C4-Phospholipids after 15 minutes of resting. Black arrow indicates phase separation in sample without C4-PL.

[047] **Figure 7** shows lace (dentelle) samples, without C4-Phospholipids (left) and with C4-Phospholipids (right).

[048] **Figure 8** shows the viscosity of emulsions prepared with C4PL and soy lecithin.

[049] **Figure 9** shows the creaming behaviour of model emulsion containing 3% oil, 3.3% milk protein and different concentration of C4PL over the duration of one hour.

[050] Some figures and text contain color representations or entities. Color illustrations are available from the Applicant upon request or from an appropriate Patent Office. A fee may be imposed if obtained from a Patent Office.

Detailed Description

Definitions

[051] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the disclosure belongs. All patents, patent applications, published applications and publications, databases, websites and other published materials referred to throughout the entire disclosure, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference to the identifier evidences the availability and public dissemination of such information.

[052] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[053] In the context of this specification, the term "about," is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[054] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[055] The term "optionally" is used herein to mean that the subsequently described feature may or may not be present or that the subsequently described event or circumstance may or may not occur. Hence the specification will be understood to include and encompass embodiments in which the feature is present and embodiments in which the feature is not present, and embodiments in which the event or circumstance occurs as well as embodiments in which it does not.

[056] As used herein, a "lipid" is any of a class of organic compounds that are or comprise fatty acids, which may be esterified or non-esterified, or their derivatives.

[057] As used herein, the term "non-polar lipid" refers to fatty acids and derivatives thereof which are soluble in organic solvents but insoluble in water and which are not charged at pH of 5-7. Non-polar lipids do not include phospholipids, galactolipids and sphingolipids, which are types of polar lipids. Typically, non-polar lipids are not soluble or very sparingly soluble in ethanol. The fatty acids may be free fatty acids and/or in an esterified form. Examples of esterified forms include, but are not limited to, triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG). Non-polar lipids also include sterols, sterol esters and wax esters. Non-polar lipids are also known as "neutral lipids" or in some contexts referred to as "oils". Non-polar lipid may be a liquid at room temperature, or a solid, depending on the degree of unsaturation of the fatty acids in the non-polar lipid. Typically, the more saturated the fatty acid content, the higher the melting temperature of the lipid.

[058] As used herein, an "oil" is a composition comprising predominantly lipid and which is a liquid at room temperature. For instance, an oil may comprise at least 75%, at least 80%, at least 85% or at least 90% lipid by weight. Typically, a purified oil comprises at least 90% triacylglycerols (TAG) by weight of the lipid in the oil, such as at least 95%, at least 96%, at least 97%, at least

98% TAG by weight. Minor components of an oil such as diacylglycerols (DAG), free fatty acids (FFA), phospholipid and sterols may be present.

[059] As used herein, the term "polar lipid" refers to amphipathic lipid molecules having a hydrophilic head and a hydrophobic tail, including phospholipids (e.g. phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, diphosphatidylglycerols), cephalins, sphingolipids (sphingomyelins and glycosphingolipids), phosphatidic acid, cardiolipin and glycoglycerolipids. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, and amino alcohols. They are generally considered to be structural lipids, playing important roles in the structure of the membranes of plants, microbes and animals. Because of their chemical structure, polar lipids exhibit a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents.

[060] As used herein, the term "fatty acid" refers to a carboxylic acid (or organic acid), often with a long aliphatic tail, either saturated or unsaturated. Unsaturated fatty acids include monounsaturated fatty acids and polyunsaturated fatty acids (PUFA). A fatty acid may be a free fatty acid (FFA) or esterified to a glycerol or glycerol-phosphate molecule, CoA molecule or other headgroup as known in the art, such as esterified as part of a polar lipid such as a phospholipid.

[061] As used herein, the term "short chain fatty acid" or "SCFA" refers to fatty acids that have a carbon-carbon bonded chain of 2, 4, 6, or 8 carbon atoms in length, such as C2:0, C4:0, C6:0 and C8:0. SCFA as used herein do not include fatty acids having 9 or more carbon atoms in their acyl chain. As used herein, the term "medium chain fatty acid" or "MCFA" refers to fatty acids that have a carbon-carbon bonded chain of between 10 and 14 carbon atoms in length, such as C10:0, C12:0, C12:1, C14:0 and C14:1. MCFA as used herein do not include fatty acids having less than 10 or more than 14 carbon atoms in their acyl chain. As used herein, the term "long chain fatty acid" or "LCFA" refers to fatty acids that have a carbon-carbon bonded chain of between 15 and 19 carbon atoms in length, such as C15:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3 fatty acids. LCFA as used herein do not include fatty acids having less than 15 or more than 18 carbon atoms in their acyl chain.

[062] Typically, the SCFA in the lipids of the present invention are saturated i.e. do not have any carbon-carbon double bonds in their acyl chain, and have an unbranched acyl chain.

[063] "Saturated fatty acids" do not contain any double bonds or other functional groups along the chain. The term "saturated" refers to hydrogen, in that all carbons (apart from the carboxylic acid [-COOH] group) contain as many hydrogens as possible.

[064] "Unsaturated fatty acids" are of similar form to saturated fatty acids, except that one or more alkene functional groups exist along the chain, with each alkene substituting a singly-bonded "-CH2-CH2-" part of the chain with a doubly-bonded "-CH=CH-" portion (that is, a carbon double bonded to another carbon). The two next carbon atoms in the chain that are bound to either side of the double bond can occur in a cis or trans configuration, preferably in the cis configuration.

[065] As used herein, the term "monounsaturated fatty acid" refers to a fatty acid which comprises at least 12 carbon atoms in its carbon chain and only one alkene group (carbon-carbon double bond) in the chain. Monounsaturated fatty acids include C12:1Δ9, C14:1Δ9, C16:1Δ9, C18:1Δ9and

C18:1Δ11. As used herein, the terms "polyunsaturated fatty acid" or "PUFA" refer to a fatty acid which comprises at least 12 carbon atoms in its carbon chain and at least two alkene groups (carbon-carbon double bonds). PUFA include C18:2Δ9,12 and C18:3Δ9,12,15.

[066] As used herein, "C4:0" refers to butyric acid, either esterified or non-esterified.

[067] As used herein, "C6:0" refers to caproic acid, either esterified or non-esterified.

[068] As used herein, "C8:0" refers to caprylic acid, either esterified or non-esterified.

[069] As used herein, "C10:0" refers to capric acid, either esterified or non-esterified.

[070] As used herein, "C12:0" refers to lauric acid, either esterified or non-esterified.

[071] As used herein, "C14:0" refers to myristic acid, either esterified or non-esterified.

[072] As used herein, "C15:0" refers to n-pentadecanoic acid, either esterified or non-esterified.

[073] As used herein, "C16:1" refers to palmitoleic acid, or-hexadec-9-enoic acid, either esterified or non-esterified. which is an omega-7 monounsaturated fatty acid.

[074] As used herein, "C16:0" refers to palmitic acid, either esterified or non-esterified.

[075] As used herein, "C18:0" refers to stearic acid, either esterified or non-esterified.

[076] As used herein, "C18:1Δ9", sometimes referred to in shorthand as "C18:1", refers to oleic acid.

[077] As used herein, "C18:1Δ9 trans" refers to elaidic acid.

[078] As used herein, "C18:1Δ11" refers to vaccenic acid.

[079] As used herein, "C18:2" refers to linoleic acid.

[080] As used herein, "C18:3" refers to linolenic acid, including α-linolenic acid and/or γ-linolenic acid.

[081] As used herein, "C18:3n3" refers to α-linolenic acid.

[082] As used herein, the term "total fatty acid (TFA) content" or variations thereof refers to the total amount of fatty acids in, for example, a lipid composition or microorganism cell, on a weight basis. The TFA may be expressed as a percentage of the weight of the cell or other fraction, e.g., as a percentage of the polar lipid. Unless otherwise specified, the weight with regard to the cell weight is the dry cell weight (DCW). In an embodiment, TFA content is measured by conversion of the fatty acids to fatty acid methyl esters (FAME) or fatty acid butyl esters (FABE) and measurement of the amount of FAME or FABE by GC, using addition of a known amount of a distinctive fatty acid standard as a quantitation standard in the GC. Typically, the amount and fatty acid composition of lipids or compositions comprising only fatty acids in the range of C10-C24 are determined by conversion to FAME, whereas lipids or compositions comprising fatty acids in the range of C4-C10 are determined by conversion to FABE. TFA therefore represents the weight of just the fatty acids, not the weight of the fatty acids and their linked moieties in the lipid or composition.

[083] The term "phospholipid", as used herein, refers to an amphipathic molecule, having a hydrophilic head and a hydrophobic tail, that has a glycerol backbone esterified to a phosphate

"head" group and two fatty acids which provide the hydrophobic tail. The phosphate group can be modified with simple organic molecules such as choline, ethanolamine or serine. Due to their charged headgroup at neutral pH, phospholipids are polar lipids, having some solubility in solvents such as ethanol in addition to solvents such as chloroform. Phospholipids are a key component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic. Well known phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylglycerol (PG) and cardiolipin.

[084] "Triacylglyceride" or "triglyceride' or "TAG" is a glyceride in which the glycerol is esterified with three fatty acids which may be the same (e.g. as in tri-olein) or, more commonly, different. All three of the fatty acids may be different, or two of the fatty acids may be the same and the third is different. In the Kennedy pathway of TAG synthesis, DAG is formed, and then a third acyl group is esterified to the glycerol backbone by the activity of DGAT.

[085] "Diacylglyceride" or "DAG" is glyceride in which the glycerol is esterified with two fatty acids which may be the same or, preferably, different. As used herein, DAG comprises a hydroxyl group at a *sn*-1,3 or *sn*-2 position, and therefore DAG does not include phosphorylated molecules such as PA or PC. In the Kennedy pathway of DAG synthesis, the precursor *sn*-glycerol-3-phosphate (G3P) is esterified to two acyl groups, each coming from a fatty acid coenzyme A ester, in a first reaction catalysed by a glycerol-3-phosphate acyltransferase (GPAT) at position *sn*-1 to form LysoPA, followed by a second acylation at position *sn*-2 catalysed by a lysophosphatidic acid acyltransferase (LPAAT) to form phosphatidic acid (PA). This intermediate is then de-phosphorylated by PAP to form DAG.

[086] "Monoacylglyceride" or "MAG" is glyceride in which the glycerol is esterified with one fatty acid. As used herein, MAG comprises a hydroxyl group at an *sn*-1/3 (also referred to as *sn*-1 MAG or 1-MAG or 1/3-MAG) or a *sn*-2 position (also referred to herein as 2-MAG), and therefore MAG does not include phosphorylated molecules such as PA or PC.

[087] As used herein, the term "triacylglycerol (TAG) content" or variations thereof refers to the amount of TAG in the cell or in the extracted lipid, according to the context. TAG content can be calculated using techniques known in the art such as the sum of glycerol and fatty acyl moieties using a relation: % TAG by weight = 100x ((41x total mol FAME/3)+(total g FAME- (15x total mol FAME)))/g, where 41 and 15 are molecular weights of glycerol moiety and methyl group, respectively (where FAME is fatty acid methyl esters).

[088] The present invention is predicated, at least in part, on the inventors' surprising finding that that particular lipids containing esterified fatty acids, especially short chain fatty acids, in profiles differing from those found in dairy milk, nonetheless exhibit dairy milk-like qualities, such as one or more of flavour, creaminess and mouthfeel. Such compositions accordingly find potential use in various applications, in particular in the preparation of food and beverage products, especially dairy substitute products which are free from or contain reduced amounts of animal-derived dairy ingredients.

Phospholipids

[089] The present inventors have developed lipids comprising phospholipids, wherein the phospholipids contain esterified short chain fatty acids, such as C4:0, C6:0 and/or C8:0 fatty acids, that exhibit desirable dairy like qualities, such as flavour, creaminess and mouthfeel. In the Examples described herein, C4:0-containing phospholipids were prepared from lecithin and tributyrin by enzymatic transesterification, and the resulting phospholipids containing elevated amounts of incorporated C4:0 fatty acids provided advantageous physical and sensory properties. Indeed, as determined by the present inventors, although the lipids of the present invention do not mimic the fatty acid profile of dairy lipids (i.e. contain ratios and amounts of SCFA (such as C4:0) and other fatty acids that differ significantly from the ratios and amounts found in dairy lipids, such as dairy phospholipids), the lipids of the present disclosure nonetheless exhibit similar physical properties to dairy lipids, including the ability to form lipid droplets, the ability to form particles of similar size to dairy lipids, and the ability to act as an emulsifier. Moreover, the lipids of the present disclosure can impart dairy-like (including cheese-like, cream-like and/or butter-like) taste, aroma and/or mouthfeel to compositions, foods, and beverages. In particular examples, the lipids are modified lecithin lipids, and comprise the same classes of phospholipid and the same fatty acids, with the exception that the lipids of the present disclosure comprise short chain fatty acids (e.g. C4 and/or C6) where unmodified lecithin lipids do not.

[090] The lipid provided herein comprise phospholipids that comprise esterified short chain fatty acids typically also comprise one or more other esterified fatty acids, including but not limited to one or more of C10, C12, C14, C15, C16, C17, C18, C19, C20, C22 and C24 fatty acids. In some instances, the phospholipids provided herein that comprise esterified short chain fatty acids typically also comprise one or more esterified C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1 (such as C18:1Δ9, C18:1Δ9 trans, and/or C18:1Δ11), C18:2, C18:3, C19:0, C20:0, C20:4, C20:5, C22:0, C22:1, C22:0, C22:6, C24:0 and C26:0. In particular examples, the phospholipids provided herein comprise esterified short chain fatty acids such as C4:0, C6:0 and/or C8:0, and also comprise a C16 and/or a C18 esterified fatty acid, such as one or more or all of C16:0, C18:0, C18:1, C18:2 and C18:3. In one example, the phospholipids comprise esterified C4:0, C16:0, C18:1 and C18:2; C4:0, C16:0, C18:0, C18:1 and C18:2; C4:0, C16:0, C18:1, C18:2 and C18:3; or C4:0, C16:0, C18:0, C18:1, C18:2 and C18:3.

[091] In one aspect, the present disclosure relates to a lipid comprising phospholipids, wherein said lipid or phospholipid comprises lipid or phospholipid esterified fatty acids, wherein said lipid or phospholipid esterified fatty acids comprise at least 2 mol% short chain fatty acids (i.e. C2, C4, C6, and/or C8 fatty acids, such as C2:0, C4:0, C6:0 and/or C8:0 fatty acids). In some embodiments, said esterified fatty acids comprise at least 1 mol% short chain fatty acids, for example at least 2 mol% short chain fatty acids, for example at least 3 mol% short chain fatty acids, for example at least 4 mol% short chain fatty acids, for example at least 5 mol% short chain fatty acids, for example at least 6 mol% short chain fatty acids, for example at least 7 mol% short chain fatty acids, for example at least 8 mol% short chain fatty acids, for example at least 9 mol% short chain fatty acids, for example at least 10 mol% short chain fatty acids, for example at least 11 mol% short chain fatty acids, for example at least 12 mol% short chain fatty acids, for example at least 13 mol% short chain fatty acids, for example at least 14 mol% short chain fatty acids, for example at least 15 mol%

short chain fatty acids, for example at least 16 mol% short chain fatty acids, for example at least 17 mol% short chain fatty acids, for example at least 18 mol% short chain fatty acids, for example at least 19 mol% short chain fatty acids, for example at least 20 mol% short chain fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% short chain fatty acids.

[092] In some embodiments, the lipids or phospholipids comprise up to about 80 mol%, for example up to about 70 mol%, for example up to about 60 mol%, for example up to about 50 mol%, for example up to about 45%, for example up to about 40 mol%, for example up to about 35 mol% esterified short chain fatty acids, for example up to about 30 mol%, for example up to about 25 mol% esterified short chain fatty acids, for example up to about 20 mol% esterified short chain fatty acids.

[093] In some embodiments, the lipids or phospholipids comprise from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol%; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids, or from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified short chain fatty acids.

[094] In particularly preferred embodiments, said lipids or phospholipids comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 1 or at least 2 mol% C4 and/or C6 fatty acids, or a combination thereof. In some embodiments, said esterified fatty acids comprise at least 3 mol% C4 and/or C6 fatty acids, for example at least 4 mol% C4 and/or C6 fatty acids, for example at least 5 mol% C4 and/or C6 fatty acids, for example at least 7 mol% C4 or C6 fatty acids, for example at least 8 mol% C4 and/or C6 fatty acids, for example at least 8 mol% C4 or C6 fatty acids, for example at least 10 mol% C4 and/or C6 fatty acids, for example at least 11 mol% C4 and/or C6 fatty acids, for example at least 12 mol% C4 and/or C6 fatty acids, for example at least 13 mol% C4 and/or C6 fatty acids, for example at least 14 mol% C4 and/or C6 fatty acids, for example at least 15 mol% C4 and/or C6 fatty acids, for example at least 20 mol% C4 and/or C6 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% C4 and/or C6 fatty acids. In preferred embodiments, said C4 and/or C6 fatty acids are C4:0 and/or C6:0 fatty acids.

[095] In some embodiments, the lipids or phospholipids comprise up to about 80 mol%, for example up to about 70%, for example up to about 60%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to about 35%, for example up to

about 30%, for example up to about 25% esterified C4 and/or C6 fatty acids, or a combination thereof. In preferred embodiments, the phospholipids comprise up to about 25 mol% esterified C4 and/or C6 fatty acids, or a combination thereof.

[096] In some embodiments, the lipids or phospholipids comprise from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 3.5 mol% to about about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 4 mol% to about about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; from about 4.5 mol% to about about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof; or from about 5 mol% to about about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 or C6 fatty acids, or a combination thereof.

[097] In some embodiments, said lipids or phospholipids comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 1 or 2 mol% C2 fatty acids. In some embodiments, said esterified fatty acids comprise at least 3 mol% C2 fatty acids, for example at least 4 mol% C2 fatty acids, for example at least 5 mol% C2 fatty acids, for example at least 7 mol% C2 fatty acids, for example at least 10 mol% C2 fatty acids, for example at least 15 mol% C2 fatty acids, for example at least 20 mol% C2 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% C2 fatty acids. In preferred embodiments, said C2 fatty acids are C2:0 fatty acids.

[098] In some embodiments, the lipids or phospholipids comprise up to about 60 mol%, for example up to about 50 mol%, for example up to about 45 mol%, for example up to about 40%, for example up to about 30 mol%, for example up to about 25 mol% esterified C2 fatty acids. In preferred embodiments, the phospholipids comprise up to about 25 mol mol% esterified C2 fatty acids.

[099] In some embodiments, the lipids or phospholipids comprise from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty

acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids; or from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C2 fatty acids.

[100] In some preferred embodiments, said lipids or phospholipids comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 1, 1.5 or 2 mol% C4 fatty acids. In some embodiments, said esterified fatty acids comprise at least 2.5% C4 fatty acids, for example at least 3 mol% C4 fatty acids, for example at least 3.5 mol% C4 fatty acids, for example at least 4 mol% C4 fatty acids, for example at least 4.5 mol% C4 fatty acids, for example at least 5 mol% C4 fatty acids, for example at least 5.5 mol% C4 fatty acids, for example at least 6 mol% C4 fatty acids, for example at least 7 mol% C4 fatty acids, for example at least 10 mol% C4 fatty acids, for example at least 15 mol% C4, for example at least 20 mol% C4 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% C4 fatty acids. In preferred embodiments, said C4 fatty acids are C4:0 fatty acids.

[101] In some embodiments, the lipids or phospholipids comprise up to about 60 mol%, for example up to about 50%, for example up to about 45 mol%, for example up to about 40 mol%, for example up to about 30%, for example up to about 25 mol% esterified C4 fatty acids. In preferred embodiments, the phospholipids comprise up to about 25 mol% esterified C4 fatty acids.

[102] In some embodiments, the lipids or phospholipids comprise from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids; from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids, from about 5.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C4 fatty acids, from about 6 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol%

esterified C4 fatty acids. In some embodiments, the phospholipids comprise from about 2 mol% to about 60 mol% esterified C4 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 5 mol% to about 35 mol%, for example from about 5 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C4 fatty acids.

[103] In some embodiments, said lipid or phospholipids comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 1, 1.5 or 2 mol% C6 fatty acids. In some embodiments, said esterified fatty acids comprise at least 2.5 mol% C6 fatty acids, for example at least 3 mol% C6 fatty acids, for example at least 3.5 mol% C6 fatty acids, for example at least 4 mol% C6 fatty acids, for example at least 4.5 mol% C6 fatty acids, for example at least 5 mol% C6 fatty acids, for example at least 5.5 mol% C6 fatty acids, for example at least 6 mol% C6 fatty acids, for example at least 7 mol% C6 fatty acids, for example at least 10 mol% C6 fatty acids, for example at least 15 mol% C6 fatty acids, for example at least 20 mol% C6 fatty acids. In preferred embodiments, said C6 fatty acids are C6:0 fatty acids.

[104] In some embodiments, the lipid or phospholipids comprise up to about 60 mol%, for example up to about 50%, for example up to about 45 mol%, for example up to about 40 mol%, for example up to about 30 mol%, for example up to about 25 mol% esterified C6 fatty acids. In preferred embodiments, the phospholipids comprise up to about 25 mol% esterified C6 fatty acids.

[105] In some embodiments, the lipid or phospholipids comprise from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; or from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids. In some embodiments, the phospholipids comprise from about 2 mol% to about 60 mol% esterified C6 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C6 fatty acids.

[106] In some embodiments, said lipid or phospholipids comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 1, 1.5 or 2 mol% C8 fatty acids. In some embodiments,

said esterified fatty acids comprise at least 3 mol% C8 fatty acids, for example at least 4 mol% C8 fatty acids, for example at least 5 mol% C8 fatty acids, for example at least 7 mol% C8 fatty acids, for example at least 10 mol% C8 fatty acids, for example at least 15 mol% C8 fatty acids, for example at least 20 mol% C8 fatty acids. In preferred embodiments, said C8 fatty acids are C8:0 fatty acids.

[107] In some embodiments, the lipid or phospholipids comprise up to about 60 mol%, for example up to about 50 mol%, for example up to about 45 mol%, for example up to about 40 mol%, for example up to about 30 mol%, for example up to about 25 mol% esterified C8 fatty acids. In preferred embodiments, the phospholipids comprise up to about 25 mol% esterified C8 fatty acids.

[108] In some embodiments, the lipid or phospholipids comprise from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids; or from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C8 fatty acids. In some embodiments, the phospholipids comprise from about 2 mol% to about 60 mol% esterified C8 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C8 fatty acids.

[109] Typically, the phospholipids present in lipids of the present disclosure further comprise esterified LCFA. In preferred embodiments, the phospholipids comprise C16 or C18 esterified fatty acids. In preferred embodiments, the phospholipids comprise both C16 and C18 esterified fatty acids. In some embodiments, the C18 is C18:0, C18:1, C18:2 or C18:3, or any combination thereof. Thus, in some examples, the phospholipids comprise C4 (such as in an amount set forth above), C16 (e.g. C16:0) and C18 (e.g. C18:0, C18:1, C18:2 and/or C18:3). In some examples, the phospholipids comprise C4 and/or C6 (such as in an amount set forth above), C16 (e.g. C16:0) and C18 (e.g. C18:0, C18:1, C18:2 and/or C18:3). In some examples, the phospholipids comprise C4, C6 and/or C8 (such as in an amount set forth above), C16 (e.g. C16:0) and C18 (e.g. C18:0, C18:1, C18:2 and/or C18:3).

[110] Said LCFA may be either saturated, monounsaturated or polyunsaturated. In preferred embodiments, the LCFA esterified in the phospholipids comprise both saturated and unsaturated LCFA, especially saturated, monounsaturated and polyunsaturated LCFA.

[111] Typically, LCFA esterified in the phospholipids have unbranched acyl chains.

[112] In some embodiments, the phospholipids described above comprising SCFA further comprise esterified C16 fatty acids. In some embodiments, the phospholipids comprise at least about 3 mol%, for example at least about 5 mol%, for example at least about 7 mol%, for example at least about 10 mol%, for example at least about 12 mol% esterified C16 fatty acids, for example at least about 13 mol%, for example at least about 14 mol%, preferably C16:0 fatty acids. In some embodiments, the phospholipids comprise at least about 15 mol%, for example at least about 16 mol%, for example at least about 17 mol%, for example at least about 18 mol%, for example at least about 19 mol%, for example at least about 20 mol% esterified C16 fatty acids, preferably C16:0 fatty acids. In preferred embodiments, the phospholipids comprise at least about 10 mol%, 15mol% or at least about 20 mol% esterified C16 fatty acids, preferably C16:0 fatty acids.

[113] In some embodiments, the phospholipids comprise up to about 60 mol%, for example up to about 50%, for example up to about 45 mol%, for example up to about 40 mol% esterified C16 fatty acids, preferably C16:0 fatty acids. In some embodiments, the phospholipids comprise up to about 35%, for example up to about 30 mol%, for example up to about 25 mol%, for example up to about 20 mol%, for example up to about 17 mol% esterified C16 fatty acids, preferably C16:0 fatty acids. In preferred embodiments, the phospholipids comprise up to about 45 mol%, or up to about 30mol% esterified C16 fatty acids, preferably C16:0 fatty acids.

[114] In some embodiments, the phospholipids comprise from about 3 mol% to about 60 mol% esterified C16 fatty acids, for example from about 5 mol% to about 55 mol%, for example from about 10 mol% to about 50 mol%, for example from about 10 mol% to about 40 mol% , for example from about 10 mol% to about 30 mol% esterified C16 fatty acids. In some embodiments, the phospholipids comprise from about 10 mol% to about 60 mol% esterified C16 fatty acids, for example from about 15 mol% to about 55 mol%, for example from about 15 mol% to about 50 mol%, for example from about 15 mol% to about 40 mol% , for example from about 15 mol% to about 30 mol%, for example from about 15 mol% to about 25 mol%, esterified C16 fatty acids. In some embodiments, the phospholipids comprise from about 20 mol% to about 60 mol%, for example from about 20 mol% to 55 mol%, for example from about 20 to 45mol% esterified C16 fatty acids. In some alternative embodiments, the phospholipids comprise from about 3 mol% to about 30 mol%, for example from about 5 mol% to about 25 mol%, for example from about 10 mol% to about 20 mol% esterified C16 fatty acids.

[115] In some embodiments, the 16 fatty acids include refers C16:0 and/or C16:1 fatty acids. In some embodiments, "C16 fatty acids" refers to C16:0 fatty acids.

[116] In some embodiments, the phospholipids comprise esterified C18 fatty acids. In some embodiments, the phospholipids comprise both unsaturated and saturated C18 fatty acids, preferably unsaturated, monounsaturated and polyunsaturated C18 fatty acids.

[117] In some embodiments, the phospholipids comprise at least about 30 mol%, for example at least about 35 mol%, for example at least about 40 mol%, for example at least about 45 mol%, for example at least about 50 mol% total C18 esterified fatty acids. In preferred embodiments, the phospholipids comprise at least about 50 mol% C18 esterified fatty acids.

[118] In some embodiments, the phospholipids comprise up to about 85 mol%, for example up to about 80 mol%, for example up to about 77 mol%, for example up to about 75 mol% total C18 esterified fatty acids. In preferred embodiments, the phospholipids comprise up to about 75 mol% C18 esterified fatty acids.

[119] In some embodiments, the phospholipids comprise from about 30 mol% to about 85 mol%, 90 mol% or 90 mol%, for example from about 35 mol% to about 85 mol%, for example from about 35 mol% to about 80 mol%, for example from about 40 mol% to about 80 mol%, for example from about 40 mol% to about 77 mol%, for example from about 40 mol% to about 75 mol%, for example from about 50 mol% to about 75 mol% C18 esterified fatty acids. In some examples, the phospholipids comprise from about 25 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol% C18 esterified fatty acids. In some examples, the phospholipids comprise from about 30 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol% C18 esterified fatty acids. In some examples, the phospholipids comprise from about 35 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol% C18 esterified fatty acids. In some examples, the phospholipids comprise from about 40 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol% C18 esterified fatty acids.

[120] In some embodiments, the phospholipids comprise at least about 0.5 mol% or 1 mol%, for example at least about 1.5 mol%, for example at least about 2 mol%, for example at least about 2.5 mol%, for example at least about 3 mol%, for example at least about 3.5 mol%, for example at least about 4 mol% C18:0 esterified fatty acids. In preferred embodiments, the phospholipids comprise at least about 2 mol% C18:0 esterified fatty acids.

[121] In some embodiments, the phospholipids comprise up to about 20 mol%, for example up to about 15 mol%, for example up to about 12 mol%, for example up to about 10 mol% C18:0 esterified fatty acids. In some embodiments, the phospholipids comprise up to about 7 mol%, for example up to about 5 mol%, for example up to about 4 mol% C18:0 esterified fatty acids. In preferred embodiments, the phospholipids comprise up to about 10 mol% C18:0 esterified fatty acids.

[122] In some embodiments, the phospholipids comprise from about 0.5 mol% or 1 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 1.5 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 2 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 2.5 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20mol%, for example from about 3 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol% C18:0 esterified fatty

acids. In some examples, the phospholipids comprise from about 2 mol% to about 10 mol% C18:0 esterified fatty acids.

[123] In some embodiments, the phospholipids comprise at least about 2 mol%, for example at least about 3 mol%, for example at least about 4 mol%, for example at least about 5 mol%, for example at least about 6 mol%, for example at least about 7 mol%, for example at least about 8 mol% C18:1 esterified fatty acids. In preferred embodiments, the phospholipids comprise at least about 5 mol% C18:1 esterified fatty acids.

[124] In some embodiments, the phospholipids comprise up to about 70, 60, 50, 40 or 30 mol%, for example up to about 25 mol%, for example up to about 20 mol%, for example up to about 17 mol%, for example up to about 15 mol% C18:1 esterified fatty acids. In preferred embodiments, the phospholipids comprise up to about 15 mol% C18:1 esterified fatty acids.

[125] In some embodiments, the phospholipids comprise from about 2 mol% to about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or 65 mol%, for example from about 5 mol% to about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or 65 mol%, for example from about 2 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%, for example from about 2 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%, for example from about 4 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%, for example from about 5 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%, for example from about 6 mol% to about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%, for example from about 7 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%, for example from about 5 mol% to about 25 mol%, for example from about 5 mol% to about 20 mol%, for example from about 5 mol% to about 15 mol% C18:1 esterified fatty acids.

[126] In some embodiments, the phospholipids comprise at least about 5 mol%, for example at least about 10 mol%, for example at least about 15 mol%, for example at least about 20 mol%, for example at least about 25 mol% C18:2 esterified fatty acids. In some embodiments, the phospholipids comprise at least about 30 mol%, for example at least about 35 mol%, for example at least about 40 mol%, for example at least about 45 mol% C18:2 esterified fatty acids. In preferred embodiments, the phospholipids comprise at least about 20 mol% C18:2 esterified fatty acids.

[127] In some embodiments, the phospholipids comprise up to about 75 mol%, for example up to about 70 mol%, for example up to about 65 mol%, for example up to about 60 mol%, for example up to about 55 mol% C18:2 esterified fatty acids. In preferred embodiments, the phospholipids comprise up to about 60 mol% C18:2 esterified fatty acids.

[128] In some embodiments, the phospholipids comprise from about 5 mol% to about 75 mol%, for example from about 10 mol% to about 70 mol%, for example from about 15 mol% to about 70 mol%, for example from about 20 mol% to about 70 mol%, for example from about 25 mol% to about 70 mol%, for example from about 20 mol% to about 60 mol%, for example from about 30 mol% to about 60 mol% C18:2 esterified fatty acids. In some embodiments, the phospholipids comprise from about 30 mol% to about 75 mol%, for example from about 30 mol% to about 70 mol%, for example from about 30 mol% to about 65 mol%, for example from about 35 mol% to

about 65 mol%, for example from about 40 mol% to about 60 mol% C18:2 esterified fatty acids. In some embodiments the phospholipids comprise from about 20 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%;from about 25 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; from about 30 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; from about 35 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; or from about 40 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol% C18:2 esterified fatty acids.

[129] In some embodiments, the phospholipids comprise at least about 0.5 mol% or at least about 1 mol%, for example at least about 1.5 mol%, for example at least about 2 mol%, for example at least about 2.5 mol%, for example at least about 3 mol%, for example at least about 3.5 mol%, for example at least about 4 mol% C18:3 esterified fatty acids. In some embodiments, the phospholipids comprise at least about 2 mol% C18:3 esterified fatty acids.

[130] In some embodiments, the phospholipids comprise up to about 20 mol%, for example up to about 17 mol%, for example up to about 15 mol%, for example up to about 12 mol% C18:3 esterified fatty acids. In some embodiments, the phospholipids comprise up to about 8 mol%, for example up to about 7 mol% C18:3 esterified fatty acids. In preferred embodiments, the phospholipids comprise up to about 15 mol% C18:3 esterified fatty acids.

[131] In some embodiments, the phospholipids comprise from about 1 mol% to about 20 mol% C18:3 esterified fatty acids, or from about 1 mol% to about 20 mol%, for example from about 2 mol% to about 17 mol%, for example from about 2 mol% to about 15 mol% C18:3 esterified fatty acids. In some embodiments, the phospholipids comprise from about 0.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%, for example from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol% C18:3 esterified fatty acids.

[132] In some embodiments, the phospholipids comprise n-3 fatty acids. In some embodiments, the esterified C18:3 fatty acids comprise or consist essentially of C18:3n3 fatty acids. In some examples, at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the esterified C18:3 fatty acids are esterified C18:3 fatty acids.

[133] In particular examples, the phospholipids comprise C16:0, C18:0, C18:1, C18:2 and C18:3. In some examples, the lipid or phospholipid esterified fatty acids comprise from about 5 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 25 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 1 mol% to about 10 mol% C18:3 fatty acids.

[134] In some embodiments, the phospholipids may comprise esterified medium or long chain fatty acids other than those discussed above, for example C20 fatty acids such as C20:4. Each of these (alone or in combination) may be present in amounts of at least about 2 mol%, for example

at least about 3 mol%, for example at least about 4 mol%, for example at least about 5 mol%, for example at least about 10 mol%, for example at least about 15 mol%, for example at least about 20 mol%; and/or up to about 75 mol%, for example up to about 70 mol%, for example up to about 65 mol%, for example up to about 60 mol%, for example up to about 55 mol%, for example up to about 50 mol%, for example up to about 45 mol%, for example up to about 40 mol%, for example up to about 35 mol%, for example up to about 30 mol%, for example up to about 25 mol%. The presence of particular fatty acids may depend, for example, on the methods used for preparing the phospholipids, such as the starting lecithin used to prepare the phospholipids or the microorganism used to produce the phospholipids.

[135] As will be familiar to a person skilled in the art, the fatty acid composition of the phospholipids may be measured by conversion of the fatty acids to fatty acid methyl esters (FAME) or fatty acid butyl esters (FABE) and measurement of the amount of FAME or FABE by GC, using addition of a known amount of a distinctive fatty acid standard as a quantitation standard in the GC.

[136] In one embodiment, the phospholipids comprise at least two, at least three or all four of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS), wherein one or more or each comprises one, two or more of C2:0, C4:0, C6:0 and C8:0, or one or both of C4:0 and C6:0, for example comprising C4:0. In particular examples, the phospholipids comprise no synthetic phospholipids, i.e. comprise only natural phospholipids selected from one or more of PC, PE, PI, PS, phosphatidylglycerol (PG), and phosphatidic acid (PA). Synthetic phospholipids are natural phospholipids (e.g. PC, PE, PI, PS, PG or PA) that have been modified in the non-polar and/or polar regions, and include, but are not limited to, 1,2-Dipalmitoyl-sn-glycero-3-phosphorylethanolamine (DPPE), 1,2-Dipalmitoyl-sn-glycero-3-phosphatidic acid sodium salt (DPPA), 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG), 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE), 1,2-Distearoyl-sn-Glycero-3-Phosphatidic acid Na salt (DSPA), 1,2-Distearoyl-sn-glycero-3-phosphorylglycerol sodium salt (DSPG) 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP), L-a-phosphatidylcholine (HSPC) 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). In other examples, the phospholipids comprise one or more synthetic phospholipids.

[137] The phospholipids may comprise from about 15% to about 60% PC or from about 20% to about 55% PC, such as from about 20% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 25% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 26% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 27% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 28% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 29% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 30% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 31% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 32% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%,

53%, 54% or 55% PC, from about 33% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, from about 34% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC, or from about 35% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54% or 55% PC (w/w or by abundance).

[138] The phospholipids may comprise from about 10% to about 50% PE or from about 15% to about 45% PE, such as from about 15% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 16% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 17% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 18% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 19% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 20% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 21% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 22% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 23% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 24% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE, from about 25% to about 30%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45% PE (w/w or by abundance).

[139] In some examples, the phospholipid comprises PC and PE, at a ratio of from about 1:1 to about 5:1 PC:PE, such as from about 1:1 to about 2:1, from about 1:1 to about 3:1, from about 1:1 to about 4:1, or from about 1:1 to about 5:1.

[140] The phospholipids may comprise from about 1% to about 40% PI or from about 1% to about 55% PI, such as from about 1% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, or 50% PI, from about 2% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35% or 40% PI, from about 3% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%,PI, from about 4% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35% or 40% PI, from about 5% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35% or 40% PI, from about 6% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35% or 40% PI, from about 7% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35% or 40% PI, or from about 10% to about 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35% or 40% PI (w/w or by abundance).

[141] The phospholipids may comprise from about 1% to about 25% PS or from about 1% to about 25% PS, such as from about 1% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% PS, from about 2% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% PS, from about 3% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% PS, from about 4% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%,

21%, 22%, 23%, 24% or 25% PS, from about 5% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% PS, or from about 6% to about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% PS (w/w or by abundance).

[142] The phospholipid esterified C4 may occur on any class on phospholipid, including one or more of PC, PE, PS, PI and PA. In some examples, over 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95% or more of the C4 is on PC and PE classes of phospholipid. In some examples, at least 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 58%, 49%, or 50% of the esterified C4 is on PC, and/or at least 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 58%, 49%, or 50% of the esterified C4 is on PE. In some examples, from about 30% to about 60%, such as from about 30% to about 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% or 60%; from about 35% to about 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% or 60%; from about 40% to about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% or 60; or from about 45% to about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% or 60%,of phospholipid esterified C4 is on PC and/or is on PE. In some embodiments, PC comprising esterified C4 make up at least 4%, 4.5%, 5%, 5.5%, 6%, or 6.5% of the phospholipids by abundance. In some embodiments, PE comprising esterified C4 make up at least 4%, 4.5%, 5%, 5.5%, 6%, or 6.5% of the phospholipids by abundance. In some embodiments, PI comprising esterified C4 make up at least 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1% of the phospholipids by abundance.

[143] It will be appreciated that the precise composition of the phospholipid esterified fatty acids may depend on the manner in which the phospholipids are prepared, and the precise composition of the phospholipids may be tailored accordingly. For example, in embodiments, wherein phospholipids are prepared by transesterification of a phospholipid-containing lecithin with a triacylglyceride or diacylglyceride comprising esterified short chain fatty acids, the composition of the resulting phospholipids will depend on that of the starting lecithin, as well as the identity of the triacylglyceride or diacylglyceride used. In some embodiments, the source of starting phospholipids is a plant lecithin, such as soy lecithin, sunflower lecithin, rapeseed lecithin or cottonseed lecithin. In such examples, the resulting phospholipids typically comprise the esterified short chain fatty acids (e.g. C4:0, C6:0 and/or C8:0) and at least two or more of C16:0, C18:0, C18:1, C18:2 and C18:3. In some examples, the resulting phospholipids comprise the esterified short chain fatty acids (e.g. C4:0, C6:0 and/or C8:0) and C16:0, C18:0, C18:1, C18:2 and C18:3. In other examples, the source of starting phospholipids egg yolk lecithin. In some examples, the resulting phospholipid when using egg yolk lecithin comprises the esterified short chain fatty acids (e.g. C4:0, C6:0 and/or C8:0) and C16:0, C18:0, C18:1, C18:2 and C20:4. In other embodiments, the source of starting phospholipids is fungal or yeast lecithin (e.g. from Mortierella sp. such as M. alpina, Saccharomyces sp. such as S. cerevisiae, Yarrowia sp. such as Y. lipolytica, Pichia sp. such as P. pastoris, Candida sp. such as C. rugosa, Aspergillus sp. such as A. niger, Cryptococcus sp. such as C. curvatus, Lipomyces sp. such as L. starkeyi, Rhodosporidium sp. such as R. toruloides, Rhodotorula sp. such as R. glutinis and Trichosporon sp. such as T. fermentans. In some embodiments, the triacylglyceride is tributyrin.

Monoacylglycerides (MAG)

[144] The present inventors have further found that lipids comprising monoacylglycerides, wherein the monoacylglycerides contain esterified short chain fatty acids, such as C4:0 fatty acids, exhibit desirable dairy like qualities, such as flavour, creaminess and mouthfeel. In the Examples described herein, C4:0-containing monoacylglycerides were prepared from tributyrin by enzymatic conversion, and the resulting monoacylglycerides containing esterified C4:0 fatty acids provided advantageous sensory properties.

[145] Accordingly, in one aspect, the present disclosure provides a lipid comprising monoacylglycerides, said monoacylglycerides comprising monoacylglyceride esterified fatty acids, wherein said monoacylglyceride esterified fatty acids comprise at least 2 mol% short chain fatty acids (i.e. C2, C4, C6, and/or C8 fatty acids, such as C2:0, C4:0, C6:0 and/or C8:0 fatty acids). In some embodiments, said monoacylglyceride esterified fatty acids comprise at least 3 mol% short chain fatty acids, for example at least 4 mol% short chain fatty acids, for example at least 5 mol% short chain fatty acids, for example at least 7 mol% short chain fatty acids, for example at least 8 mol% short chain fatty acids, for example at least 9 mol% short chain fatty acids, for example at least 10 mol% short chain fatty acids, for example at least 11 mol% short chain fatty acids, for example at least 12 mol% short chain fatty acids, for example at least 13 mol% short chain fatty acids, for example at least 14 mol% short chain fatty acids, for example at least 15 mol% short chain fatty acids, for example at least 16 mol% short chain fatty acids, for example at least 17 mol% short chain fatty acids, for example at least 18 mol% short chain fatty acids, for example at least 19 mol% short chain fatty acids, for example at least 20 mol% short chain fatty acids. In preferred embodiments, said monoacylglyceride esterified fatty acids comprise at least 3 mol% short chain fatty acids.

[146] In some embodiments, the monoacylglycerides comprise up to about 60 mol%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to about 30%, for example up to about 25% esterified short chain fatty acids.

[147] In some alternative embodiments, the monoacylglycerides comprise at least about 50%, for example at least 55 mol% short chain fatty acids, for example at least 60 mol% short chain fatty acids, for example at least 65 mol% short chain fatty acids, for example at least 70 mol% short chain fatty acids, for example at least 75 mol% short chain fatty acids, for example at least 80 mol% short chain fatty acids, for example at least 85 mol% short chain fatty acids, for example at least for example at least 90 mol% short chain fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 50 mol% short chain fatty acids, especially at least about 70 mol% short chain fatty acids.

[148] In some embodiments, the monoacylglycerides comprise from about 2 mol% to about 60 mol% esterified short chain fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified short chain fatty acids.

[149] In preferred embodiments, said short chain fatty acids are saturated fatty acids.

[150] In particularly preferred embodiments, said monoacylglycerides comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 2 mol% C4 and/or C6 fatty acids, or a combination thereof. In some embodiments, said esterified fatty acids comprise at least 3 mol% C4 and/or C6 fatty acids, for example at least 4 mol% C4 and/or C6 fatty acids, for example at least 5 mol% C4 and/or C6 fatty acids, for example at least 7 mol% C4 or C6 fatty acids, for example at least 8 mol% C4 and/or C6 fatty acids, for example at least 8 mol% C4 or C6 fatty acids, for example at least 10 mol% C4 or C6 fatty acids, for example at least 11 mol% C4 and/or C6 fatty acids, for example at least 12 mol% C4 and/or C6 fatty acids, for example at least 13 mol% C4 and/or C6 fatty acids, for example at least 14 mol% C4 and/or C6 fatty acids, for example at least 15 mol% C4 and/or C6 fatty acids, for example at least 20 mol% C4 and/or C6 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% C4 and/or C6 fatty acids. In preferred embodiments, said C4 and/or C6 fatty acids are C4:0 and/or C6:0 fatty acids.

[151] In some embodiments, the monoacylglycerides comprise up to about 60 mol%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to about 30%, for example up to about 25% esterified C4 and/or C6 fatty acids, or a combination thereof. In preferred embodiments, the monoacylglycerides comprise up to about 25 mol% esterified C4 and/or C6 fatty acids, or a combination thereof.

[152] In some alternative embodiments, the monoacylglycerides comprise at least about 50%, for example at least 55 mol% esterified C4 and/or C6 fatty acids, or a combination thereof, for example at least 60 mol%, for example at least 65 mol%, for example at least 70 mol%, for example at least 75 mol%, for example at least 80 mol%, for example at least 85 mol% esterified C4 and/or C6 fatty acids, or a combination thereof, for example at least 90 mol% esterified C4 and/or C6 fatty acids, or a combination thereof. In preferred embodiments, said esterified fatty acids comprise at least 50 mol% esterified C4 and/or C6 fatty acids, or a combination thereof, especially at least about 70 mol% esterified C4 and/or C6 fatty acids, or a combination thereof.

[153] In some embodiments, the monoacylglycerides comprise from about 2 mol% to about 60 mol% esterified C4 and/or C6 fatty acids, or a combination thereof, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C4 and/or C6 fatty acids, or a combination thereof.

[154] In some preferred embodiments, said monoacylglycerides comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 2 mol% C2 fatty acids. In some embodiments, said esterified fatty acids comprise at least 3 mol% C2 fatty acids, for example at least 4 mol% C2 fatty acids, for example at least 5 mol% C2 fatty acids, for example at least 7 mol% C2 fatty acids, for example at least 10 mol% C2 fatty acids, for example at least 15 mol% C2 fatty acids, for example at least 20 mol% C2 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% C2 fatty acids. In preferred embodiments, said C2 fatty acids are C2:0 fatty acids.

[155] In some embodiments, the monoacylglycerides comprise up to about 60 mol%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to

about 30%, for example up to about 25% esterified C2 fatty acids. In preferred embodiments, the monoacylglycerides comprise up to about 25 mol% esterified C2 fatty acids.

[156] In some alternative embodiments, the monoacylglycerides comprise at least about 50%, for example at least 55 mol% esterified C2 fatty acids, for example at least 60 mol%, for example at least 65 mol%, for example at least 70 mol%, for example at least 75 mol%, for example at least 80 mol%, for example at least 85 mol% esterified C2 fatty acids, for example at least 90 mol% esterified C2 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 50 mol% esterified C2 fatty acids, especially at least about 70 mol% esterified C2 fatty acids.

[157] In some embodiments, the monoacylglycerides comprise from about 2 mol% to about 60 mol% esterified C2 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C2 fatty acids.

[158] In some preferred embodiments, said monoacylglycerides comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 2 mol% C4 fatty acids. In some embodiments, said esterified fatty acids comprise at least 3 mol% C4 fatty acids, for example at least 4 mol% C4 fatty acids, for example at least 5 mol% C4 fatty acids, for example at least 7 mol% C4 fatty acids, for example at least 10 mol% C4 fatty acids, for example at least 15 mol% C4, for example at least 20 mol% C4 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 3 mol% C4 fatty acids. In preferred embodiments, said C4 fatty acids are C4:0 fatty acids.

[159] In some embodiments, the monoacylglycerides comprise up to about 60 mol%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to about 30%, for example up to about 25% esterified C4 fatty acids. In preferred embodiments, the monoacylglycerides comprise up to about 25 mol% esterified C4 fatty acids.

[160] In some alternative embodiments, the monoacylglycerides comprise at least about 50%, for example at least 55 mol% esterified C4 fatty acids, for example at least 60 mol%, for example at least 65 mol%, for example at least 70 mol%, for example at least 75 mol%, for example at least 80 mol%, for example at least 85 mol% esterified C4 fatty acids, for example at least 90 mol% esterified C4 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 50 mol% esterified C4 fatty acids, especially at least about 70 mol% esterified C4 fatty acids.

[161] In some embodiments, the monoacylglycerides comprise from about 2 mol% to about 60 mol% esterified C4 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C4 fatty acids.

[162] In some embodiments, said monoacylglycerides comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 2 mol% C6 fatty acids. In some embodiments, said esterified fatty acids comprise at least 3 mol% C6 fatty acids, for example at least 4 mol% C6 fatty acids, for example at least 5 mol% C6 fatty acids, for example at least 7 mol% C6 fatty acids, for example at

least 10 mol% C6 fatty acids, for example at least 15 mol% C6 fatty acids, for example at least 20 mol% C6 fatty acids. In preferred embodiments, said C6 fatty acids are C6:0 fatty acids.

[163] In some embodiments, the monoacylglycerides comprise up to about 60 mol%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to about 30%, for example up to about 25% esterified C6 fatty acids. In preferred embodiments, the monoacylglycerides comprise up to about 25 mol% esterified C6 fatty acids.

[164] In some alternative embodiments, the monoacylglycerides comprise at least about 50%, for example at least 55 mol% esterified C6 fatty acids, for example at least 60 mol%, for example at least 65 mol%, for example at least 70 mol%, for example at least 75 mol%, for example at least 80 mol%, for example at least 85 mol% esterified C6 fatty acids, for example at least 90 mol% esterified C6 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 50 mol% esterified C6 fatty acids, especially at least about 70 mol% esterified C6 fatty acids.

[165] In some embodiments, the monoacylglycerides comprise from about 2 mol% to about 60 mol% esterified C6 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C6 fatty acids.

[166] In some embodiments, said monoacylglycerides comprise esterified fatty acids, wherein said esterified fatty acids comprise at least 2 mol% C8 fatty acids. In some embodiments, said esterified fatty acids comprise at least 3 mol% C8 fatty acids, for example at least 4 mol% C8 fatty acids, for example at least 5 mol% C8 fatty acids, for example at least 7 mol% C8 fatty acids, for example at least 10 mol% C8 fatty acids, for example at least 15 mol% C8 fatty acids, for example at least 20 mol% C8 fatty acids. In preferred embodiments, said C8 fatty acids are C8:0 fatty acids.

[167] In some embodiments, the monoacylglycerides comprise up to about 60 mol%, for example up to about 50%, for example up to about 45%, for example up to about 40%, for example up to about 30%, for example up to about 25% esterified C8 fatty acids. In preferred embodiments, the monoacylglycerides comprise up to about 25 mol% esterified C8 fatty acids.

[168] In some alternative embodiments, the monoacylglycerides comprise at least about 50%, for example at least 55 mol% esterified C8 fatty acids, for example at least 60 mol%, for example at least 65 mol%, for example at least 70 mol%, for example at least 75 mol%, for example at least 80 mol%, for example at least 85 mol% esterified C8 fatty acids, for example at least 90 mol% esterified C8 fatty acids. In preferred embodiments, said esterified fatty acids comprise at least 50 mol% esterified C8 fatty acids, especially at least about 70 mol% esterified C8 fatty acids.

[169] In some embodiments, the monoacylglycerides comprise from about 2 mol% to about 60 mol% esterified C8 fatty acids, for example from about 3 mol% to about 50 mol%, for example from about 3 mol% to about 45 mol%, for example from about 3 mol% to about 40 mol%, for example from about 3 mol% to about 30 mol%, for example from about 3 mol% to about 25 mol% esterified C8 fatty acids.

[170] In some preferred embodiments, lipids and compositions of the present disclosure may comprise both phospholipids and monoacylglycerides of the present disclosure.

[171] The lipid of the present disclosure may consist essentially of phospholipids and/or monoglycerides. Alternatively, in some embodiments, lipids of the present disclosure may comprise other components as part of a broader lipid component. Said other components may comprise, for example, other polar or non-polar lipids, including triglycerides or diglycerides, free fatty acids, sugars, sugar derivatives and organic acids. Said further components may be present in the product mixture obtained when preparing the monoacylglycerides and/or phospholipids of the present disclosure, such as by enzymatic processes disclosed herein. In some examples, the lipid of the present disclosure comprises at least or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% phospholipids; or at least or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% phospholipids and/or monoglycerides. In further examples, the lipid of the present disclosure comprises at least or about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% phospholipids, and less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% monoacylglycerides, such as at least or about 99%, 99.5% or 99.9% phospholipids and less than 1%, 0.5% or 0.1% monoacylglycerides. In further examples, the lipid of the present disclosure comprises at least or about 99%, 99.5% or 99.9% phospholipids and monoacylglycerides are undetectable. In further embodiments, the lipid comprises less than 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% triacylglycerides.

Compositions

[172] The present disclosure further relates to compositions comprising a lipid of the present invention together with one or more further components. In particular embodiments, the compositions are modified lecithins, such as produced by interesterification of a lecithin (such as described further herein) so as to produce a lipid of the present invention (e.g. as described above) with other components. The compositions may therefore include the modified lipid resulting from the interesterification, and also comprise other components that are present in lecithins, such as protein, carbohydrates, and/or ash.

[173] Compositions of the present disclosure may comprise one or more peptides or proteins. In one example, the protein is a protein found in lecithin (e.g. is a lecithin-derived protein) and is present in a composition of the present invention that is a modified lecithin. For example, the protein may be a soy, sunflower, rapeseed, or cottonseed protein (e.g. may be derived from soy, sunflower, rapeseed, or cottonseed lecithin). Further examples of proteins which may be present in compositions of the present invention include (but are not limited to) bioactive peptides/proteins, flavour-related peptides/proteins, soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins. In particular, compositions may include milk proteins (e.g., one or more whey proteins [e.g., a b-lactoglobulin, a a-lactalbumin, a mixture of a b-lactoglobulin and a a-lactalbumin], one or more caseins [e.g., a k-casein, a b-casein, a g-casein, a mixture of a k-casein and a b-casein, a mixture of a K-casein and a g-casein, a mixture of a b-casein and a g-casein], or any combination thereof [i.e. , a mixture of one or more whey proteins and one or more caseins]).

[174] A recombinant or native milk protein can be obtained from any mammalian species, including but not limited to cow, human, sheep, goat, buffalo, camel. Methods for extracting native milk proteins and/or producing recombinant milk proteins are disclosed in U.S. Patent 9,924,728, issued March 27, 2018; U.S. publication US20190216106, published July 18, 2019; and PCT publication WO2019213155, published November 7, 2019; which are hereby incorporated herein in their entireties.

[175] Alternatively or in addition, the compositions may comprise one or more non-milk proteins obtained from any source, as well as mixtures of non-milk proteins obtained from various sources. Non-limiting examples of suitable sources include animals, plants, algae, fungi, and bacteria.

[176] The protein present in compositions of the present invention may be a native protein, such as a native milk protein, or a recombinant or synthesised protein.

[177] Compositions of the present disclosure may comprise one or more carbohydrates. In one example, the carbohydrate is derived from lecithin and is present in a composition of the present invention that is a modified lecithin. For example, the carbohydrate may be derived from a soy, sunflower, rapeseed, or cottonseed lecithin. In other examples, the carbohydrate has been added to the lipid in the preparation of the composition.

[178] Non-limiting examples of carbohydrates include monosaccharides, disaccharides, and polysaccharides. Nonlimiting examples of monosaccharides include glucose, fructose, and dextrose. Non-limiting examples of disaccharides include maltose, lactose, and sucrose. Non-limiting examples of polysaccharides include maltodextrin, starches, flours, and edible fibers. Non-limiting examples of suitable starches include maltodextrin, inulin, fructooligosaccharides, pectin, carboxymethyl cellulose, guar gum, com starch, oat starch, potato starch, rice starch, pea starch, and wheat starch. Non-limiting examples of suitable flours include but amaranth flour, oat flour, quinoa flour, rice flour, rye flour, sorghum flour, soy flour, wheat flour, and com flour. Non-limiting examples of suitable edible fibers include bamboo fiber, barley bran, carrot fiber, citrus fiber, corn bran, soluble dietary fiber, insoluble dietary fiber, oat bran, pea fiber, rice bran, head husks, soy fiber, soy polysaccharide, wheat bran, wood pulp cellulose, and derivatives and mixtures thereof.

[179] Further examples of components which may be present in compositions of the present disclosure include nutritional supplements, prebiotics, probiotics, pro-vitamins, vitamins, minerals, antioxidants (i.e., molecules capable of slowing or preventing oxidation of other molecules), carbohydrates, and essential and semi essential amino acids (e.g., cysteine, methionine, isoleucine, leucine, phenylanine, tryptophan, valine).

[180] Non-limiting examples of vitamins include lipid soluble vitamins, water soluble vitamins, thiamin (vitamin Bl), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), vitamin B6 (pyridoxine), vitamin B12 (cobalamin), vitamin C, folate, vitamins A, vitamin D, vitamin E, vitamin K, and derivatives and mixtures thereof.

[181] Non-limiting examples of minerals include calcium, phosphorous, potassium, sodium, citrate, chloride, phosphate, magnesium, potassium, zinc, iron, molybdenum, manganese, copper, and mixtures thereof.

[182] Non-limiting examples of antioxidants include a-tocopherol (e.g., tocopherol comprised in bovine milk), low molecular weight thiols (e.g., low molecular weight thiols comprised in bovine milk), retinol (e.g., retinol comprised in bovine milk), carotenoids (e.g., carotenoids comprised in cow milk, a-carotene, b-carotene, g-carotene, lutein, zeaxanthin, astaxanthin), vitamin E, Azadirachta indica extract, riboflavin, rosemary extract, phenolic diterpenes (e.g., camosol, camosic acid) comprised in rosemary extract, sage extract, ascorbic acid (vitamin C) and its salts, lactic acid and its salts, grape residue silage, phenolic compounds (e.g., ferulic acid) comprised in grape residue silage, soybean (Glycine max) extract, isoflavones or polyphenolic compounds comprised in soybean extract, garlic (Allium sativum) extract, phenolic or flavonoid, or terpenoid compounds comprised in garlic extract, fennel (Foeniculum vulgare Mill.) extract, chamomile (Matricaria recutita L.) extract, fatty acids (e.g., alpha-lipoic acid), brown algae (e.g., Ascophyllum nodosum, Fucus vesiculosus), essential oils of green pink pepper (GEO), essential oils of mature pink pepper (MEO), green tea extract, butylated hydroxy anisole (E320), butylated hydroxy toluene (E321), polyphenols (e.g., curcumins, curcuminoids, desmethoxycurcumin (hydroxycirmamoyl feruloylmethane), bis-desmethoxycurcumin), catechins (e.g., epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, C catechin, catechins comprised in green tea extract), and derivatives and mixtures thereof.

[183] Compositions of the present disclosure may comprise one or more further bioactive agents. Said bioactive molecules may include, for example, therapeutics and neutraceuticals. Non-limiting examples of such bioactive agents include steroids, anti-oxidants, vitamins, minerals, fatty acids, amino acids, prebiotics, probiotics, bioactive peptides, proteins, enzymes, drugs, antibodies, and hormones. In some examples, the bioactive agents include one or more of bioactive peptides and proteins found in the milk fat globule membrane (MFGM). Non-limiting examples include adipophilin (ADPH), butyrophilin (BTN), mucins (e.g. mucin 1 (MUC1)), xanthine dehydrogenase/oxidase (XDH/XO), CD36, lactadherin, periodic acid Schiff III (PAS III), fatty acid-binding protein (FABP).

[184] Compositions of the present disclosure may further comprise functional agents including buffering agents, shelf life extending agents, pH and/or ionic strength adjusting agents (i.e., agents that raise or lower the pH and/or the ionic strength of a solution), preservatives, emulsifiers, plasticizers, texturing/mouthfeel agents, coloring agents, flavor/aroma agents, and sweetening agents.

[185] In some examples, the compositions of the present disclosure are modified lecithins. Such compositions typically comprise lipid (i.e. a lipid as described above), carbohydrate and protein derived from lecithin, and optionally ash. In some examples, the compositions comprise at least 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49% or 50% fat or lipid. In some examples, the total fat (oil/dry weight) of the composition is from about 30% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 35% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 40% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 42% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 44% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%,

70%, 71%, 72%, 73%, 74% or 75%; from about 45% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 46% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 48% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; or from about 50% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%. In some examples, the total fatty acids in the composition comprise about 1.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 % C4 fatty acids; from about 2 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 25, 30 or 35 % C4 fatty acids; from about 2.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 25, 30 or 35 % C4 fatty acids; from about 3 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 3.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 4 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 4.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; or from about 5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids. In some examples, the total fatty acids comprises about 70% to about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 75% to about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 80% to about 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 85% to about 90, 92, 93, 94 or 95 % long chain fatty acids; or from about 90% to about 93, 94, 95, 96, 97 or 98% long chain fatty acids. In some examples, the only lipid in the compositions is a lipid of the present disclosure, as described above. Thus, in some examples, phospholipids are present in the composition in an amount of at least 30%, 35%, 40%, 45% or 50% by weight, such as from about 30% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74% or 75% by weight, such as from about 35% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74% or 75% by weight, such as from about 40% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74% or 75% by weight, such as from about 45% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74% or 75% by weight.

[186] The compositions, such as those that are modified lecithins, may comprise from about 1 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 2 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 3 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 4 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); or from about 5 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight). In further examples, the compositions comprise from about 5% to about 20, 25, 30, 35, 40 or 50 % carbohydrate (by weight); from about 6 % to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 7% to about 20, 25, 30, 35, 40% carbohydrate (by weight); from about 8% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 9% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about

10% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); or from about 5% to about 10, 20, 25, 30, 35, 40, or 50% carbohydrate (by weight).

[187] In some embodiments, compositions comprise the lipid of the present invention, an aqueous component and a hydrophobic component.

[188] The hydrophobic component may be or comprise, for example, a non-polar lipid, for example comprising triacylglycerides.

[189] In some embodiments, the non-polar lipid is in the form of an oil. Examples of suitable oils include, but are not limited to lemon oil, sunflower oil, soybean oil, canola oil, olive oil, corn oil, peanut oil, groundnut oil, rice bran oil, coconut oil, cottonseed oil, flax seed oil, palm oil, palm kernel oil, safflower oil, soybean oil, sesame oil, amaranth oil, linseed oil, argan oil, grapeseed oil, cranberry seed oil, hazelnut oil, hemp oil, jojoba oil, macadamia oil, mustard oil, neem oil, orange oil, rapeseed oil, avocado oil, almond oil, sweet almond oil, cashew oil, castor oil, vegetable oil, walnut oil, wheatgerm oil, kukui nut oil, tamuna oil, aloe vera oil, apricot kernel oil, borage oil (from, for example Borago officionalis), camellia oil (from, for example, Camellia oleifera), cocoa butter oil , rosehip see oil, fish oils, ethyl oleate, ethyl linoleate, saturated fatty acids (such as, but not limited to, caproic acid, caprylic acid, capric acid, lauric acid, valeric acid, myristic acid, palmitic acid, stearic acid, arachidic acid), medium chain triglycerides, omega-3 fatty acids (such as, but not limited to, hexadecatrienoic acid, alpha-linolenic acid, stearidonic acid, ecosatrienoic acid, eicosapentaenoic acid, heneicosapentanoic acid, docosapentanoic acid, docosahexanoic acid, tetracosapentaenoic acid, tetracosahexanenoic acid), omega-6 fatty acids (such as, but not limited to, linoleic acid, gamma-linolenic acid, eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosadienoic acid, adrenic acid, docosapentaenoic acid, tetracosatetraenoic acid, tetracosapentaenoic acid), and/or omega-9 fatty acids (such as, but not limited to, oleic acid, eicosenoic acid, mead acid, erucic acid, nervonic acid).

[190] In particular examples, the compositions do not comprise cholesterol.

[191] In particular embodiments, the compositions are emulsified.

[192] Such compositions are particularly advantageous as they have been found to be capable of forming lipid droplet structures when prepared using phospholipids according to the present invention. Images of such lipid droplet structures obtained using phospholipids of the present disclosure are shown in Figure 3, where a hydrophobic "core" comprising triacylglycerides (Figure 3A) can be seen surrounded by outer layers of phospholipid (Figure 3B). As will be understood in the context of the present disclosure, "lipid droplet" may be understood as referring to a droplet comprising a hydrophobic core, surrounded by at least one layer comprising phospholipids. In some examples, the lipid droplets have a volume moment mean diameter (or De Brouckere mean diameter), D[4,3], of less than 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, or 8 μm, and/or or at least 1, 2, 3, 4 or 5 μm.

[193] Without wishing to be bound by theory, such lipid droplet structures are thought to be particularly advantageous in the preparation of dairy and dairy substitute products since they mimic the structure of native milk fat globules, depicted in Figure 3D; milk fat is present in milk in the form of hydrocolloid assemblies, consisting of a neutral lipid core (triacylglycerol) and a tri-layer

membrane mostly comprised of phospholipid. As shown in Figure 2, compositions of the present invention were also found to give droplet sizes similar to that of unhomogenised whole milk. It is thought that these qualities of compositions of the present disclosure, which mimic those of dairy products, contribute to the ability of the lipids and compositions of the present disclosure to mimic other aspects of dairy products such as taste, creaminess and mouthfeel.

[194] In some embodiments, the composition, such as the emulsified composition, comprises the lipids of the present disclosure such that the phospholipids are present in the composition in an amount of at least 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.011%, 0.012%, 0.013%, 0.014%, 0.015%, 0.016%, 0.017%, 0.018%, 0.019%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045% 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% 1%, 2% or 3% by weight; and/or up to 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%,10%, 11%, 12%, 13%, 15%, 16%, 17%, 18%, 19%, 20% by weight. As would be appreciated, the final amount of the phospholipids in a composition comprising lipids of the present disclosure is a factor of the dilution. In some examples, the compositions comprises from about 0.001 esterified C4 or C6 fatty acids, or a combination thereof, to about 0.01, 0.1, 1 or 10 mol% esterified C4 or C6 fatty acids, or a combination thereof; or from about 0.01 esterified C4 or C6 fatty acids, or a combination thereof, to about 0.1, 1 or 10 mol% esterified C4 or C6 fatty acids, or a combination thereof; or from about 0.1 mol% esterified C4 or C6 fatty acids, or a combination thereof, to about 1 or 10 mol% esterified C4 or C6 fatty acids, or a combination thereof; or from about 1 mol% esterified C4 or C6 fatty acids, or a combination thereof, 10 mol% esterified C4 or C6 fatty acids, or a combination thereof. In some examples, the compositions comprises from about 0.005 esterified C4 or C6 fatty acids, or a combination thereof, to about 0.05, 0.5, 5 mol% esterified C4 or C6 fatty acids, or a combination thereof; or from about 0.05 esterified C4 or C6 fatty acids, or a combination thereof, to about 0.5 or 5 mol% esterified C4 or C6 fatty acids, or a combination thereof; or from about 0.5 mol% esterified C4 or C6 fatty acids, or a combination thereof, to about 5 mol% esterified C4 or C6 fatty acids, or a combination thereof.

[195] Such lipid droplet compositions further find use as delivery vehicles, for delivering one or more components to a subject, the delivery vehicle comprising a composition of the present disclosure wherein the composition comprises lipid droplets. Said components to be delivered may, in some embodiments, be contained in the hydrophobic "core" of the lipid droplet. The lipid droplet may, in certain embodiments, provide advantages such as increased protection and stability of the delivered component from oxidation, or increased bioavailability of the components to the subject.

[196] The delivery vehicle may be suitable for delivering, for example, one or more bioactive agents, as described above.

Foods, beverages and feedstuffs

[197] Compositions of the present disclosure may be included in, or be in the form of, a food, beverage or feedstuff. Accordingly, the present invention relates to foods or beverages for human consumption or a feedstuff for animal consumption, preferably at least a food or beverage for human consumption. For purposes of the present invention, a food, beverage or feedstuff is a preparation for human or animal consumption which when taken into the body (a) serve to nourish or build up tissues or supply energy; and/or (b) maintain, restore or support adequate nutritional status or

metabolic function. Food and beverages of the invention includes nutritional compositions for babies and/or young children such as, for example, infant formula. A food, beverage or feedstuff includes foods, beverages or feedstuffs intended to be consumed in their whole form, as well as foods, beverages or feedstuffs intended to be used as ingredients in broader food, beverage or feedstuff preparations.

[198] The food or beverage for human consumption or a feedstuff for animal consumption may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, protein, carbohydrate, vitamins, and/or minerals in amounts desired for a particular use. The amounts of these ingredients will vary depending on whether the composition is intended for use with normal individuals or for use with individuals having specialized needs, such as individuals suffering from metabolic disorders and the like.

[199] Examples of suitable ingredients with nutritional value include, but are not limited to, macronutrients such as edible fats, carbohydrates and proteins. Examples of such edible fats include, but are not limited to, coconut oil, borage oil, fungal oil, black current oil, soy oil, and mono- and diglycerides.

[200] Examples of such carbohydrates include (but are not limited to): glucose, edible lactose, and hydrolyzed starch.

[201] With respect to vitamins and minerals, the following may be added to the food/beverage/feedstuff of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

[202] Additional ingredients include food-grade oils such as canola, corn, sunflower, soybean, olive or coconut oil, seasoning agents such as edible salts (e.g., sodium or potassium chloride) or herbs (e.g., rosemary, thyme, basil, sage, or mint), flavouring agents, proteins (e.g., soy protein isolate, wheat glutin, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein concentrate), emulsifiers, gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo filer or inulin), or minerals (e.g., iodine, zinc, and/or calcium).

[203] Foods, beverages and feedstuffs of the invention also can include a natural coloring agent such as turmeric or beet juice, or an artificial coloring agent such as azo dyes, triphenylmethanes, xanthenes, quinines, indigoids, titanium dioxide, red #3, red #40, blue #1, or yellow #5.

[204] Foods, beverages and feedstuffs of the invention also can include shelf life extenders such as carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

[205] The components utilized in the food, beverage or feedstuff compositions of the present invention can be of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by de novo synthesis.

[206] In some embodiments, the food, beverage or feedstuff ingredient or food, beverage or feedstuff is a dairy or egg product substitute. In some embodiments, the food, beverage or feedstuff is dairy-free or egg-free. In one embodiment, the food or feedstuff is a dairy-free milk, a dairy-free

butter, a dairy-free cheese, a dairy-free yoghurt, a dairy-free cream, a dairy-free ice cream, or a dairy-free chocolate.

[207] In some alternative embodiments, the food, beverage or feedstuff is a dairy product (e.g. comprises one or more components or ingredients from a mammal, such as a mammals such as a cow, goat, sheep or buffalo). In some embodiments, the food, beverage or feedstuff is a combination of dairy and non-dairy products, or may comprise reduced levels of one or more dairy ingredients relative to a full fairy product. In some such embodiments, the lipid or composition of the present disclosure may serve to enhance particular qualities of the food, beverage or feedstuff, for example by increasing the dairy-like flavour, creaminess or mouthfeel.

[208] For example, in some embodiments, the food, beverage or feedstuff is a milk, for example a reduced fat or skim milk, yoghurt, butter, cheese, cream, ice cream or chocolate.

[209] Thus, in some examples, the food, beverage or feedstuff is an egg or egg product, or can resemble an egg or egg product (i.e., is an egg or egg product substitute). Non-limiting examples of suitable eggs and egg products include whole egg (e.g., liquid whole egg, spray-dried whole egg, frozen whole egg), egg white (e.g., liquid egg white, spray-dried egg white, frozen egg white), egg dishes, egg soups, and mixtures made with egg whites. The food, beverage or feedstuff can be a milk or dairy product, or can resemble a milk or dairy product (i.e., is a milk substitute or dairy product substitute). Non-limiting examples of milk and dairy products include milk (e.g., whole milk [at least 3.25% milk fat], partly skimmed milk [from 1% to 2% milk fat], skim milk [less than 0.2% milk fat], cooking milk, condensed milk, flavored milk, goat milk, sheep milk, dried milk, evaporated milk, milk foam), and products obtained from milk, including but not limited to yogurt (e.g., whole milk yogurt [at least 6 grams of fat per 170 g], low-fat yogurt [between 2 and 5 grams of fat per 170 g], nonfat yogurt [0.5 grams or less of fat per 170 g], greek yogurt [strained yogurt with whey removed], whipped yogurt, goat milk yogurt, Labneh [labne], sheep milk yogurt, yogurt drinks [e.g., whole milk Kefir, low-fat milk Kefir], Lassi), cheese (e.g., whey cheese such as ricotta; pasta filata cheese such as mozzarella; semi-soft cheese such as Havarti and Muenster; medium-hard cheese such as Swiss and Jarlsberg; hard cheese such as Cheddar and Parmesan; washed curd cheese such as Colby and Monterey Jack; soft ripened cheese such as Brie and Camembert; fresh cheese such as cottage cheese, feta cheese, cream cheese, and curd), processed cheese, processed cheese food, processed cheese product, processed cheese spread, enzyme-modulated cheese; cold-pack cheese), dairy-based sauces (e.g., salad dressing, bechamel sauce, fresh sauces, frozen sauces, refrigerated sauces, shelf stable sauces), dairy spreads (e.g., low-fat spread, low-fat butter), cream (e.g., dry cream, heavy cream, light cream, whipping cream, half-and-half, coffee whitener, coffee creamer, sour cream, creme fraiche), frozen confections (e.g., ice cream, smoothie, milk shake, frozen yogurt, sundae, gelato, custard), dairy desserts (e.g., fresh, refrigerated, or frozen), butter (e.g., whipped butter, cultured butter), dairy powders (e.g., whole milk powder, skim milk powder, fat-filled milk powder (i.e., milk powder comprising plant fat in place of all or some animal fat), infant formula, protein concentrate (i.e., protein content of at least 80% by weight; e.g., milk protein concentrate, whey protein concentrate, demineralized whey protein concentrate, b-lactoglobulin concentrate, a-lactalbumin concentrate, glycomacropeptide concentrate, casein concentrate), protein isolate (i.e., protein content of at least 90% by weight; e.g., milk protein isolate, whey protein isolate,

demineralized whey protein isolate, b-lactoglobulin isolate, a-lactalbumin isolate, glycomacropeptide isolate, casein isolate), nutritional supplements, texturizing blends, flavoring blends, coloring blends), ready -to-drink or ready -to-mix products (e.g., fresh, refrigerated, or shelf stable dairy protein beverages, weight loss beverages, nutritional beverages, sports recovery beverages, and energy drinks), puddings, gels, chewables, crisps, bars (e.g., nutrition bars, protein bars), and fermented dairy products (e.g., yoghurt, cheese, sour cream, cultured buttermilk, cultured butter, cultured butter oil).

[210] In further embodiments, the food, beverage or feedstuff is a baked good or baked good mix. Suitable examples of a baked goods and mixes may include, for example, a cake, a cookie, a brownie, a muffin, a bread, a bread dough, a donut, a donut, a crust, a pastry, a cracker, a desert bar, a pancake, a waffle and a bagel, and mixes and doughs thereof.

[211] In an embodiment, the food, beverage or feedstuff has no components derived from an animal. Thus, in a preferred embodiment, at least some of the ingredients are plant material or material derived from a plant. In some embodiments, the food, beverage or feedstuff can be lactose free, soy-free, wheat-free, yeast-free, MSG-free, and/or free of protein hydrolysis products.

[212] Foods, beverages and feedstuffs described herein can be assessed using trained human panelists. The evaluations can involve eyeing, feeling, chewing, smelling and/or tasting of the product to judge product appearance, color, integrity, texture, flavour, and mouth feel, etc. Panelists can be served samples under red or under white light. A scale can be used to rate the overall acceptability or quality of the food or specific quality attributes such dairy-likeness, texture, and flavour.

[213] A food, beverage or feedstuff of the invention comprises the lipid or composition of the present invention. In some embodiments, the food, beverage or feedstuff comprises the phospholipid-comprising lipids of the present disclosure in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 1.5% or 2% by weight; and/or up to 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4%, 5% or 10% by weight. In some embodiments, the food, beverage or feedstuff comprises the composition of the present disclosure in an amount of at least 0.005%, 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% by weight; and/or up to 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20% or 25% by weight.

[214] In some examples, a beverage of the present disclosure, such as a milk (such as a non-dairy milk, dairy milk and/or reduced fat milk) and comprises lipid of the present disclosure in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% or 0.1% by weight, and up to 0.05%, 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2% or 3% by weight; or comprises a composition of the present disclosure (such as a modified lecithin of the present disclosure) in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1% or 0.2% by weight and up to 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4% or 5% by weight by weight.

[215] In some examples, the food is a cheese (such as a non-dairy cheese, dairy cheese and/or reduced fat cheese) and comprises lipid of the present disclosure in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% or 1% by weight, and up to 0.05%, 0.1% 0.2%,

0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4% or 5% by weight; or comprises a composition of the present disclosure (such as a modified lecithin of the present disclosure) in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% or 1% by weight, and up to 0.05%, 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% by weight.

[216] In some examples, the food is a chocolate (such as a non-dairy chocolate or dairy chocolate) and comprises lipid of the present disclosure in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% or 0.1% by weight, and up to 0.05%, 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2% or 3% by weight; or comprises a composition of the present disclosure (such as a modified lecithin of the present disclosure) in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1% or 0.2% by weight and up to 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4% or 5% by weight by weight.

[217] In some examples, the food or beverage is a coffee creamer and comprises lipid of the present disclosure in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% or 1% by weight, and up to 0.05%, 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4% or 5% by weight; or comprises a composition of the present disclosure (such as a modified lecithin of the present disclosure) in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% or 1% by weight, and up to 0.05%, 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% by weight.

[218] Also provided is a method of producing a food product, beverage product or feedstuff as described herein, comprising combining a lipid or composition of the present invention with one or more additional consumable ingredients.

[219] In a further aspect, provided is a use of a lipid or composition as described herein to produce a food, beverage or feedstuff.

Processes of preparation

[220] The compositions and lipids described herein may be prepared by any suitable method.

[221] In some embodiments, the lipid is provided by esterification, such as transesterification.

[222] In certain embodiments, esterification, including transesterification, may comprise contacting a source of phospholipids with a) a source of short chain fatty acids desired to be incorporated into the phospholipids, especially C4, C6 and/or C8 fatty acids, especially C4 fatty acids, for example a triacylglyceride or diacylglyceride comprising esterified short chain fatty acid acids or free short chain fatty acids, and b) an enzyme suitable for effecting transesterification of the phospholipid with said short chain fatty acids, for a time and under conditions sufficient to effect such transesterification. Optionally, the resulting phospholipids can be treated further to remove all or essentially all of the free fatty acids, triacylglyceride and/or diacylglyceride. The resulting purified lipid composition may comprise at least or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% phospholipids.

[223] Transesterification may be effected by one or more enzymes. In some embodiments, the enzyme is a lipase, such as a phospholipase and/or a TAG lipase. In some examples, the enzyme has both phospholipase and TAG lipase activities. In other examples, two enzymes, a phospholipase

and a TAG lipase are used. Examples of suitable enzymes include Lecitase ultra (Novozyme) and Lipase, immobilized on Immobead 150 from *Thermomyces lanuginosus* (76546, Sigma Aldrich), or Lypozyme TL IM (Novozyme). In some embodiments, the enzyme is immobilised.

[224] The source of phospholipid to be transformed by transesterification may be, for example, a lecithin, for example a plant lecithin (e.g. a crop plant lecithin) such as a soy lecithin, canola lecithin, sunflower lecithin, cottonseed lecithin, or rapeseed lecithin. Lecithins are complex mixtures comprising lipid comprising phospholipid, protein, and carbohydrate, derived from egg yolk or the membrane of plant or animal cells. Thus, the lecithins may comprise proteins and carbohydrate (e.g. at least 1%, 2%, 3%, 4%, 5%, 67%, 7%, 8%, 9%, 10% w/w or more protein and/or carbohydrate) that are derived from the source of the lecithin, such as proteins and carbohydrate derived from soy, canola, sunflower, cottonseed or rapeseed. The lipid of the lecithin may comprise triacylglycerides, which can be removed, or mostly removed, by a de-oiling process, such that the lecithin comprises less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% triacylglycerides. Lecithins also include fractioned lecithins, such as alcohol fractionated lecithins. The present disclosure contemplates using any type of lecithin, including crude lecithin, liquid lecithin, deoiled lecithin, fractionated lecithin, high phosphatidylcholine (PC) fraction lecithin, acetylated lecithin, hydrolyzed lecithin, hydroxylated lecithin, lecithin comprising modified phospholipids, and combinations of any thereof. Generally, the phospholipids are present in the lecithin at around 20% to 70% w/w, such as at least 20%, 25%, 30%, 35%, 40%, 45% or 50% and/or no more than 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 71%, 72%, 73%, 74%, or 70% w/w.

[225] Typically, the phospholipid in the lecithin comprises PC and PE, at a ratio of from about 1:1 to about 5:1 PC:PE, such as from about 1:1 to about 2:1, from about 1:1 to about 2.5:1, from about 1:1 to about 3:1, from about 1:1 to about 3.5:1, from about 1:1 to about 4:1, from about 1:1 to about 4.5:1, or from about 1:1 to about 3:1. In some examples, the PC is present in the phospholipid at an amounts of 20% to 75%, such as from 20% to 40%, 45%, 50%, 55%, 60%, 65% 70% or 75%, or from 25% to 40%, 45%, 50%, 55%, 60%, 65% 70% or 75%. In some examples, the PE is present in the phospholipid at an amount of 15% to 60%, such as from 15% to 30%, 35%, 40%, 45%, 50%, 55% or 60, or from 25% to 40%, 45%, 50%, 55%, 60%, 65% 70% or 75%, or from 15% to 45%. Typically the phospholipids also comprise PI, PS and/or PA.

[226] Lecithins such as plant lecithins typically comprise C16 (e.g. C16:0) and C18 (e.g. C18:0, C18:1, C18:2 and C18:3) fatty acids. In some examples, the lecithin is low oleic acid lecithin. In other examples the lecithin is egg yolk lecithin, fungus lecithin or yeast lecithin.

[227] The source of short chain fatty acids to be incorporated into the phospholipids may be, for example, a triglyceride comprising the short chain fatty acid, such as tributyrin (available from Sigma Aldrich, W222305), tricaproin, tricaprylin, and medium-chain triacylglycerols (MCT) having C6, C8, C10 and/or C12 fatty acids. In other examples, the short chain fatty acids are provided as free fatty acids, e.g. free butyric acid or free caproic acid.

[228] In some embodiments, lipids of the present invention, for example monoacylglycerides disclosed herein, may be prepared by enzymatic transformation of an alternative lipid. For example, a triglyceride or diglyceride comprising esterified short chain fatty acids may be contacted with an

enzyme suitable for effecting conversion of the triacylglyceride or diacylglyceride to a monoacylglyceride, for a time and under conditions sufficient for effecting such conversion.

[229] In some embodiments, the starting triglyceride comprises C4 or C6 esterified fatty acids, for example C4 fatty acids. For example, the triglyceride may be tributyrin or tricaproin. Advantages of triglyceride such as tributyrin or tricaproin compared to diacylglyceride or monoacylglyceride with C4 or C6 include that there are three C4 or C6 acyl chains available in the triglyceride for incorporation into the phospholipid, and also that the tributyrin or tricaproin are less polar that the diacylglyceride or monoacylglyceride with C4 or C6.

[230] Suitable enzymes include lipases, for example Lipase, immobilized on Immobead 150 from *Thermomyces lanuginosus* (76546, Sigma Aldrich), or Lypozyme TL IM(Novozyme) X, or Lecitase-Ultra, (Novozyme). In some embodiments, the enzyme is immobilised.

[231] In embodiments of the invention that involve transesterification of phospholipid-containing lecithin, the molar ratio of lecithin to the source of short chain fatty acids may be any suitable ratio for effective transesterifications. In some examples, the source of short chain fatty acids is tributyrin and the mol ratio of lecithin to tributyrin is from about 1:1 to about 1:10, such as from about 1:1, 1:1.5, 1:2, or 1:2.5 to about 1:3, 1:3.5, 1:4, 1:4.5, 1:5, 1:5.5, 1:6, 1:6.5, 1:1.7, 1:7.5, 1:8, 1:8.5, 1:9, 1:9.9 or 1:10. In some examples, the mol ratio is about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 or 1:10. The concentration of enzyme may be, for example, at least or about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 25%, or 30%, such as from about 5% to about 25%, or from about 7% to about 22%. The time of reaction may be, for example, at least or about 12hr, 18 hr, 24 hr, 30 hr, 36 hr, 42 hr, 48 hr, 54 hr, 60 hr, 66 hr or 72 hr or more. In some examples, the time of reaction is from about 24 hr to about 72 hr, such as from about 36 hr to about 60 hr, such as from about 42 hr to about 54 hr. In some examples, the transesterification conditions include a mol ratio of lecithin to tributyrin of from about 1:1 to about 1:7 and a reaction time of from about 42 hr to about 54 hr.

[232] In some embodiments, monoacylglycerides disclosed herein, comprising esterified SCFA, may be prepared by way of enzymatic synthesis from glycerol and short chain fatty acids, such as butyric acid (Yang *et al*., 2003).

[233] Alternatively, the lipids disclosed herein may be prepared by other suitable methods, such as by culturing a microorganism genetically modified to produce said lipids, or produced by way of mutagenesis. For example, a microorganism may be genetically modified to produce increased amounts of phospholipids/polar lipids and/or increased amounts of SCFA esterified in phospholipids. Thus, the microorganism may comprise one or more genetic modifications providing for: synthesis of, or increased synthesis of, one or more SCFA fatty acids; an increase in total fatty acid synthesis and/or accumulation in the microorganism; an increase in total polar lipid synthesis and/or accumulation in the microorganism; a decrease in TAG synthesis and/or accumulation in the microorganism, or an increase in TAG catabolism, such as an increase in TAG lipase activity; or a reduction in catabolism of total fatty acids. Suitable microorganisms are known in the art and include bacteria (e.g. Acinetobacter baylyi ADP1(MT), Alcanivorax sp. such as Alcanivorax borkumensis SK2, Gordonia sp. such as DG, Mycobacterium sp. such as Mycobacterium tuberculosis H37Rv, Nocardia sp. such as Nocardia globerula 432, Rhodococcus sp. such as Rhodococcus opacus PD630, and

Streptomyces sp. such Streptomyces coelicolor TR0958 or TR0123.), yeast (e.g. Saccharomyces sp. such as Saccharomyces cerevisiae , Yarrowia sp. such as Yarrowia lipolytica , Pichia sp. such as Pichia pastoris, Candida sp. such as Candida rugosa, Aspergillus sp. such as Aspergillus niger, Cryptococcus sp. such as Cryptococcus curvatus, Lipomyces sp. such as Lipomyces starkeyi , Rhodosporidium sp. such as Rhodosporidium toruloides Y4, Rhodotorula sp. such as Rhodotorula glutinis and Trichosporon sp. such as Trichosporon fermentans) and other fungi (e.g. Cunninghamella sp. such as Cunninghamella echinulate, Mortierella sp. such as Mortierella isabellina or Mortierella alpina, Mucorales sp. such as Mucorales fungi and Trichoderma sp. such as Trichoderma harzianum Q2-37).

[234] The genetic modifications may include the introduction of an exogenous polynucleotide, a mutation or a deletion of a gene or regulatory sequence, or any other known genetic modification. Suitable techniques for genetically modifying microorganisms are well known to those in the art. For example, suitable recombinant DNA techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present). Reference is also made to international patent application no. PCT/AU2022/050177, the disclosure of which is incorporated herein in its entirety.

[235] Other suitable methods for preparation of the lipid of the present disclosure include fermentation of biomass under conditions suitable for production of the lipid.

[236] In some examples, the methods described above comprise further purification or enrichment steps, so as to, for example, increase the purity of the phospholipids and/or remove other lipid or fatty acid species (e.g. MAG, DAG, TAG and/or free fatty acids). In one example, the lipids are subjected to acetone or ethanol precipitation or extraction. This process can be repeated once, twice, three times or more. In further examples, the lipids are processed to enrich phospholipids esterified with short chain fatty acids, such as C2, C4, C6, and/or C8 fatty acids. In particular examples, the resulting lipid composition comprises at least or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% phospholipids.

[237] The present disclosure further relates to lipids obtained by the above processes.

[238] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Example 1 – Preparation of C4-lipids

a) Preparation of C4-phospholipid (Lipase batch 1)

[239] C4-containing phospholipids (C4-PL) were generated by interesterification of lecithin powder (Soy; Emulsification, The Melbourne Food Ingredient Depot) and Tributyrin (Sigma Aldrich, W222305) using Lipase, immobilized on Immobead 150 from *Thermomyces lanuginosus* (76546, Sigma Aldrich). One to three mol ratios of the two substrates, lecithin powder and tributyrin were measured into 50mL falcon tubes in triplicates. Soy lecithin powder was hydrated using ultra turrax with reverse osmosis (RO) water to prepare one molar solution. Measured volumes of tributyrin were added to the falcon tubes containing lecithin solution and homogenised with ultra turrax to prepare reaction mixtures. Enzyme amounts of 10, 20 and 40 wt% were measured into three sterile Erlenmeyer flasks. Premade reaction mixtures were added to the flasks containing enzyme and incubated at 45°C with gentle shaking at 500rpm for 18hrs. Reaction mixtures were centrifuged at 5000rpm for 15min at 4°C. The reaction mixture formed 3 distinctive layers; top aqueous layer, middle lecithin rich layer and bottom tributyrin rich layer. All three layers were fractionated using TLC, methylated, and analysed on GCMS to assess C4:0 incorporation percentage and identified the C4-PL enriched layer. The layer enriched with C4-PL was methylated and total fatty acid content (TFA) was analysed. TFA analysis results are in table 1 below. A second aliquot was silylated (TMS) and analysed on GCMS to identify the C4-containing MAG and DAG impurities in the final freeze-dried product. TMS results are in table 3 below. Freeze dried C4-PL product was used to assess physical properties of the product. Results are given under Example 2 below.

[240] In order to assess the C4 incorporation into pure C4-PL, an aliquot of crude C4-PL product was washed with acetone 3 times. The acetone insoluble fraction and acetone soluble fraction were dried down, methylated and analysed on GCMS to assess to C4 incorporation into PL and contribution of C4 from other sources respectively. Lipid analysis results are given in Table 2 below.

Lipid fractionation by thin layer chromatography

[241] To separate unreacted tributyrin, di-butyrin, free butyric acid and polar lipids in the total reaction mixture, 100µL aliquots of resulting top aqueous layer (Layer 1), middle lecithin layer (Layer 2) and bottom oil layer (Layer 3) were diluted in 100µL of Hexane and 10µL were fractionated on thin layer chromatography (TLC) plates (Silica gel 60; Catalog No. 1.05626.0001, MERCK, Darmstadt, Germany) using hexane:diethylether:acetic acid (70/30/1 v/v/v) as the solvent system. A reference standard TLC 18-6A containing TAG, DAG, FFA and MAG (Nu-Chek Prep Inc, USA) was run in an adjacent lane to identify the different lipid spots. After the chromatography, the plates were sprayed with a primuline (Catalog No. 206865, Sigma, Taufkirchen, Germany) solution prepared at a concentration of 5 mg/100 ml in acetone:water (80/20 v/v) and lipid bands visualised under UV light. The silica with the lipid from each spot was scraped off and transferred to a tube. Silica scrapings were derivatised using methylation and analysed on GCMS.

Lipid derivatisation to fatty acid methyl esters (FAME)

[242] For analysis by GCMS or GCFID, fatty acid methyl esters (FAME) were prepared from freeze dried C4-PL product or TLC fractionated PL scrapings by treatment with 0.75 ml 1 N methanolic-HCl (Sigma Aldrich, Catalog No. 90964) in a 2 ml glass vial having a PTTE-lined screw cap at 80°C for 2

h. A known amount of heptanoic acid (Nu-Chek Prep, Inc., Catalog No. N-7-A, Waterville, MN, USA) dissolved in toluene was added to each sample before the treatment as an internal standard for quantification. After the vials were cooled, 0.3 ml of RO water and 0.2 ml hexane were added and the mixtures vortexed for 5 min. The mixture was centrifuged at 1700 g for 5 min and the upper, hexane phase containing the FAME was analysed by GCMS. Alternatively, lipid extract was derivatised to convert the lipids to fatty acid methyl esters (FAMEs) by mixing 25 µL of sample extract with 25 µL of Methprep II (0.2N methanolic solution of m-trifluoromethylphenyl trimethylammonium hydroxide) in 250 µL conical glass inserts in a GC vial, followed by the incubation at 35 oC at 750 rpm for 30 min. An aliquot of 25µL of Nu check GLC-413 standard (2.5 mg/mL, Nu check) was prepared the same way. Derivatised samples were then run on the GCFID.

C4-PL freeze dried product silylation (TMS)

[243] A known amount of freeze dried C4-PL product and heptanoic was weighed into 300µL glass insert. A known amount of heptanoic acid standard was aliquoted into the insert and dried under Nitrogen. The glass insert was transferred into 2mL Agilent crimp cap amber vial and crimped with a magnetic crimp cap. Derivatisation of the sample was automated on 8890GC coupled to a 5977C MS (Agilent technologies, Santa Clara, United States) with Gerstel MPS2 (GERSTEL GmbH & Co. KG, Germany). 30µL aliquots of BSTFA +10% TMCS (catalogue number TS-38840, Thermofisher Scientific) were aliquoted into the samples. Samples were moved to the agitator and incubated and mixed at 45°C for 45min. Samples were moved back to the rack cooled down for 10min and 1µL was injected into GC.

Analysis and quantification of FAME by GCMS

[244] The individual FAMEs were identified and quantified by GCMS using an 8890 GC coupled to a 5977C MS (Agilent technologies, Santa Clara,United States) with a 30 m J&W DB-FastFAME column (0.25 mm inner diameter, 0.25 µm film thickness), a split/splitless injector and an Gerstel MPS2 (GERSTEL GmbH & Co. KG, Germany) autosampler, and electron impact single quadrupole mass spectrometer. Samples were injected in split mode (5:1 ratio) at an oven temperature of 50°C. Helium was used as the carrier gas at a constant flow of 1.0 ml/min. The column temperature was programmed for 50°C for 1 min, increasing to 160°C at 10°C/min, holding for 0 min and reaching 210°C at 3°C/min, then holding at 210°C for 0 min. The injector temperature was set at 250°C. MS detection was obtained in electron impact mode (70 eV) on full scan monitoring (m/z 50-500 amu). Spectra are recorded at 2.91 scans per second. The transfer line and electron ionization (EI) ion source were set at 280°C and 230°C respectively while the quadrupole at 150°C. The mass spectrometer was tuned using tris(perfluorobutyl)amine (CF43) according to the manufacturer's specified protocol. FAME peaks were identified based on retention times of FAME standard (GLC-413; NuChek Inc., USA). Peaks were identified, deconvoluted and integrated with Agilent Technologies MassHunter Unknowns Analysis (Version 12.0, Palo Alto, California, USA) based on the response of the known amount of C7:0-ME internal standard. The resultant data provide the fatty acid composition on a weight basis, with percentages of each fatty acid (weight %) in a total fatty acid content of 100%. These percentages on a weight basis could readily be converted to percentages on a molar basis (mol%) based on the known molecular weight of each fatty acid.

Qualitative analysis of TMS derivatives by GCMS

[245] The TMS derivatives including free fatty acids, SCFA and LCFA containing MAG, Dibutyrin, Sugars, Organic acids and others were identified and semi quantitated by GCMS using an 8890 GC coupled to a 5977C MS (Agilent technologies, Santa Clara,United States) with a 25 m Agilent J&W VF-5ms column (0.25 mm inner diameter, 0.25 µm film thickness), a split/splitless injector and an Gerstel MPS2 (GERSTEL GmbH & Co. KG, Germany) autosampler, and electron impact single quadrupole mass spectrometer. Samples were injected in split mode (10:1 ratio) at an oven temperature of 70°C. Helium was used as the carrier gas at a constant flow of 1.0 ml/min. The column temperature was programmed for 70°C for 1 min, increasing to 325°C at 7°C/min, holding for 5 min at the end. The injector temperature was set at 250°C. MS detection was obtained in electron impact mode (70 eV) on full scan monitoring (m/z 50-500 amu). Spectra are recorded at 2.91 scans per second. The transfer line and electron ionization (EI) ion source were set at 280°C and 230°C respectively while the quadrupole at 150°C. The mass spectrometer was tuned using tris(perfluorobutyl)amine (CF43) according to the manufacturer's specified protocol. Peaks were identified, deconvoluted and integrated with Agilent Technologies MassHunter Unknowns Analysis (Version 12.0, Palo Alto, California, USA). Unknown mass spectra were identified using NIST20 database. Match factor was set to 80. Peak areas were normalised to the total peak areas of the chromatogram and sample weight.

TFA analysis on GCFID

[246] Samples were analysed on 8890 GC (Agilent technologies) with a 30 m J&W DB-FastFAME column (0.25 mm inner diameter, 0.25 µm film thickness). An aliquot of 1 µL of samples were injected in split mode (10:1 ratio) at an oven temperature of 50 °C. Hydrogen was used as the carrier gas at a constant flow of 1.0 mL/min. The column temperature was programmed at 50°C for 0.5 min, increasing to 60°C at 2.5°C/min, holding at 60 °C for 1 min, followed by ramping to 194°C at 30°C/min, holding at 194 °C for 3.1 min, then finally reaching 240°C at 7°C/min and holding for 0.36 min. The injector temperature was set at 240 °C. FID was operated at 280 °C with air flow at 400mL/min, Hydrogen fuel flow at 30mL/min and Nitrogen makeup flow at 10mL/min. Data rate was set to 50Hz.

[247] FAME peaks were identified based on retention times of fatty acid standard (GLC-413). Peaks were integrated and quantified with Agilent Technologies Open Lab (Version 3.5, Palo Alto, California, USA) based on the response of the known amount of C17:0 internal standard mix. The resultant data provide the fatty acid composition on a weight basis, with percentages of each fatty acid (weight %) in a total fatty acid content of 100%. These percentages on a weight basis could readily be converted to percentages on a molar basis (mol%) based on the known molecular weight of each fatty acid.

Assessment of bound and free C4 by HPLC

[248] C4 bound in MAG, DAG and TAG, and in free form was analysed using an HPLC Agilent 1260 Infinity II (Agilent technologies, United States) equipped with UV detector and a Poroshell 120 EC C18 column (100×4.6 mm; 2.7µm). The detector was set to record absorbance at 222 nm and 210 nm.

[249] C4 sample extract was prepared by adding 200 mg sample into 1 mL MeOH in an Eppendorf vial. Sample was vortexed, followed by a 5 min centrifugation at 10,000 rpm. The supernatant (top layer) was filtered through a 0.2 µm nylon membrane filter (Biomed Scientific) prior to HPLC analysis. An aliquot of 0.1 – 10 µL of samples were injected into the HPLC at an oven temperature of 35 °C. Gradient elution was used at a constant flow of 0.35 mL/min. The column was initially eluted using mobile phase containing water: ACN: IPA (67.2: 28.8: 4.0 v:v:v) for 3 min, followed by a gradient elution using mobile phase ACN:IPA (60:40 v:v) to ACN:IPA (40:60 v:v) from 3.1 to 30.0 min. The column was finally eluted using Water: ACN: IPA (67.2: 28.2: 4.0 v:v:v) from 30.1 mins and hold 5.9 mins. The total run time is 36 minutes, followed by an addition of 1 min post run delay. Peaks were identified and quantified based on the retention times and known concentrations of the four standards (butyric acid, monobutyrin, dibutyrin and tributyrin) using Agilent Technologies Open Lab (Version 3.2, Palo Alto, California, USA). C4 bound to PL was then calculated by subtraction of the total C4 (determined from the fatty acid profile analysis) to the sum of C4 free and C4 bound in MAG, DAG and TAG.

Lipase batch 1 results

Table 1: µmol% per total µmol of FAME of C4-PL crude product.

	Fatty acid mol% C4-PL crude product					
	C4:0	C16:0	C18:0	C18:1	C18:2	C18:3
TLC separated PL fraction	53.6	11.9	0.0	3.7	29.8	0.9
Final C4-PL product	46.1	13.2	0.0	6.9	29.9	3.9

Table 2: µmol% per total µmol of FAME of C4-PL acetone washed product.

	Fatty acid mol % acetone washed C4-PL sample					
	C4:0	C16:0	C18:0	C18:1	C18:2	C18:3
Pure C4-PL (Acetone insoluble)	3.5	24.5	4.7	9.1	51.1	5.0
Other C4 (FFA/MG/DG) (Acetone soluble)	72.3	3.7	0.9	2.9	17.0	1.6

Table 3: Peak area % of other components of crude C4-PL product.

Compound	**Peak Area %**
Glycerides/Free fatty acids	
Butyric acid	7%
2-Mono-isobutyrin	10%
1-Mono-isobutyrin	35%
1,2-Diisobutyrin	6%
1,3-Diisobutyrin	10%
Tributyrin	1%
Palmitic Acid	0%
9,12-Octadecadienoic acid (Z,Z)-	1%
Oleic Acid, (Z)-	0%
Stearic acid	0%

Sugars/Sugar derivatives	
Glycerol	5%
Chiro-Inositol	1%
Myo-Inositol	0%
Sucrose	12%
D-Trehalose	1%
Organic acids	
Malic acid	1%
Phosphate	0%
Citric acid	5%
Glucaric acid	0%
Other	
Triethylene glycol	0%
Heptaethylene glycol	0%
L-5-Oxoproline	0%

b) C4-PL Lypozyme batch 1 and batch 2 preparation

[250] A composition containing C4-phospholipid (C4-PL) was prepared from lecithin powder **(**Soy; Emulsification, The Melbourne Food Ingredient Depot) by interesterification with tributyrin (Sigma Aldrich, W222305), reverse osmosis (RO) water and Lypozyme TL IM from Thermomyces lanuginosus (Novozyme). One to three mol ratios of the two substrates, lecithin powder and tributyrin were mixed in a sterilised food grade vacuum pouch (VS-C1625B, Chamber vacuum sealer bags, Pro-line) and mixed well.10% of substrate v/w Hexane was added to mixture to improve solubility. 20% and 10% of substrate w/w enzyme was finally added to batch 1 and 2 respectively and gently mixed. Vacuum pouches were sealed and placed at a set temperature of 45°C at 50rpm for 48 hours. The reaction mixture was transferred into a sterilised beaker and diluted with RO water in 1:3 volume ratio (Reaction mixture:Water). Diluted mixture was transferred into 1L centrifuge bottle centrifuged at 4000rpm at 4°C for 15min. Supernatant was transferred into a 1L Schott bottle. The liquid product (supernatant) was washed with food-grade acetone in 1:5 ratio (product: Acetone) and centrifuged to discard solvents. The pellet was further rinsed twice with ice cold acetone and centrifuged to discard solvent. The final product was oven dried for a minimum of 4 hours at 30°C to remove residual solvents. The product was stored at (-20°C) in food grade freezer and clearly labelled with production date. Samples were tested for solvent residue level i.e acetone and hexane, and found to have a residual acetone level below 0.1 mg/kg and a residual hexane level below 20 mg/kg.

[251] The lipid content of the product was analysed by methylating a known amount of product followed by analysis on GCMS. Other fatty acids and components were analysed by TMS method on GCMS. Lipid analysis and other fatty acids and component results are given in Tables 4 and 5 below:

Table 4

µmol% per total µmol of FAME

Sample	**C4:0**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3N3**	% oil/DW
Lypozyme batch 2	5.2	39.0	8.1	10.2	26.7	10.8	53.4
Lypozyme batch 1	21.9	15.2	3.4	9.5	45.5	4.5	53.8

[252] Other fatty acids and components were detected in minor percentages as shown in Table 5 below:

Table 5

	Lypozyme Batch 2	**Lypozyme Batch 1**
Glycerides/Free fatty acids		
1,3-Diisobutyrin	0%	0%
1-Mono-isobutyrin	0%	1%
1-Monolinolein	12%	7%
1-Monopalmitin	2%	1%
2-linoleoylglycerol	4%	2%
2-Mono-isobutyrin	0%	0%
2-Palmitoylglycerol	0%	0%
9,12-Octadecadienoic acid (Z,Z)	14%	11%
Glycerol monostearate	0%	0%
Octadecanoic acid	2%	2%
Oleic Acid, (Z)	5%	10%
Palmitic Acid	10%	8%
Sugars/Sugar derivatives		
D-Chiro-Inositol	0%	1%
Disaccharide	1%	1%
Disaccharide	4%	9%
Galactinol	1%	0%
Glucopyranose	3%	3%
Glycerol	2%	0%
Glycerol-3-phosphate	8%	11%
Inositol, myo	0%	8%
O-Methyl-inositol	1%	2%
Raffinose	1%	1%
Saccharic acid	0%	1%
Sucrose	1%	1%

	Lypozyme Batch 2	Lypozyme Batch 1
Organic acids		
Citric acid	15%	7%
Malic acid	3%	1%
Others		
2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)-4,5-dihydrooxazole Results	2%	0%
Oxazole, 4,5-dihydro-2-pentadecyl	2%	1%
Unknown	2%	8%
Unknown	3%	2%
Unknown	0%	0%

c) Preparation of C4-monoglyceride

[253] A composition containing C4-monoglyceride (C4-MAG) was prepared from tributyrin (Sigma Aldrich, W222305), lecitase-ultra (Novozyme) and RO water. A mixture of 1:1 volume ratio of tributyrin and RO water were mixed with 20% v/v lecitase ultra and the mix was incubated at 37°C for 48 hours at 50rpm. The resulting product was centrifuged at 4000rpm at 4°C for 10min. The aqueous top layer was mixed with 1:1 v/v RO water and centrifuged again at 4000rpm at 4°C for 10min to remove tributyrin. Top aqueous layer containing the product was transferred to a fresh Schott bottle and stored at (-20°C) in a food grade freezer and was clearly labelled with production date. The product was shipped at (4°C) to an external service provider (Inspired Ingredients) for freeze-drying. One aliquot of freeze dried product was methylated and analysed on GCMS for the fatty acid composition and second aliquot of the product was silylated and analysed on GCMS for free fatty acids, MAG, DAG and sugar analysis.

[254] Total lipid analysis was carried out by methylation on GCMS. Results are shown in Tables 6 and 7 below.

Table 6

µmol% per total µmol of FAME		
	C4:0 mol %	% oil/DW
C4-MG/DG freeze dried product	100%	1.5

Table 7

Product purity & Total Fatty Acid C4-MG/DG liquid product

Compound	**Purity**
C4:0	0.1%
Glycerol	1.3%
C4-MG	3.8%

C4-DG	4.9%
Sorbitol/Sugar	89.8%
Total	100.0%

*Sorbitol is the enzyme stabilizer. Concentration unknown in the CoA.

d) Preparation of C6-PL

A composition containing C6-phospholipid (C6-PL) was prepared from lecithin powder (Soy; Emulsification, The Melbourne Food Ingredient Depot) by interesterification with Hexanoic acid (W255900, Sigma Aldrich), Hexane and Lypozyme TL IM from Thermomyces lanuginosus (Novozyme). Two combinations of 1:10 and 1:20 mol ratios of the two substrates, lecithin powder and Hexanoic acid were mixed in 50mL falcon tubes and mixed well.10% of substrate v/w Hexane was added to mixture to improve solubility. 10% of substrate w/w enzyme was finally added and gently mixed. Reaction mixes were placed at a set temperature of 45°C at 50RPM for 48 hours. Reactants were centrifuged at 4000rpm at 4°C for 15min. The product was separated from the enzyme pellet and transferred to a fresh falcon tube and washed with food-grade acetone in 1:5 ratio (product: Acetone) and centrifuged to discard solvents. The pellet was further rinsed twice with ice cold acetone and centrifuged to discard solvent. The resulting pellet was oven dried at 30°C for minimum 3 hours until acetone evaporated. An aliquot of the dried product was methylated and analysed on GCMS for lipid composition. Results of lipid composition are shown in Table 8 below.

Table 8. µmol% per total µmol of FAME of C6-PL product

	µmol% per total µmol of FAME						
	C6:0	C16:0	C18:0	C18:1	C18:1iso	C18:2	C18:3
C6-PL (mol ratio 1:10)	6.49	20.62	4.28	12.25	1.21	49.90	5.26
C6-PL (mol ratio 1:20)	6.52	21.46	3.81	11.30	0.97	51.37	4.58

"C18:1iso" includes C18:1Δ9 and CC18:1Δ11

e) C4-PL Lypozyme batch 4 preparation

[255] A composition containing C4-phospholipid (C4-PL) was prepared from lecithin powder (Soy; Emulsification, The Melbourne Food Ingredient Depot) by interesterification with tributyrin (Sigma Aldrich, W222305), and Lypozyme TL IM from *Thermomyces lanuginosus* (Novozyme). One to three mol ratios of the two substrates, lecithin powder and tributyrin, were mixed in a sterilised food grade vacuum pouch (VS-C1625B, Chamber vacuum sealer bags, Pro-line) and mixed well. 10% of substrate v/w Hexane (Supelco, ACS grade) was added to the mixture to improve solubility. 20% of substrate w/w enzyme was finally added, and the reaction mixture was gently mixed. Vacuum pouches were sealed and placed at a set temperature of 45°C at 75 rpm for 72 hours. The reaction mixture was transferred into a sterilised beaker and diluted with RO water in 1:1 volume ratio (Reaction mixture: Water). Diluted mixture was transferred into 1L centrifuge bottle and centrifuged at 3000rpm at 4°C for 15min. Supernatant was transferred into a 1L Schott bottle. The liquid product (supernatant) was washed with food-grade acetone at 4°C in 1:5 v:v ratio (product: Acetone) and centrifuged to discard solvents. The pellet was further rinsed three times with ice cold acetone and centrifuged to discard solvent. The final product was freeze dried under vacuum, beginning at -20°C and increasing to 60°C over a 24 hour period. The resulting powder was then resuspended in RO

water a ratio of 15:2 (mL:g) of water to product, and mixed well before a second round of freeze drying at the same conditions. The final product was stored at (-20°C) in food grade freezer and clearly labelled with production date. Samples were tested for solvent residue level i.e. acetone and hexane. The results showed that the sample had a residual acetone level below 10 mg/kg and a residual hexane level below 20 mg/kg.

[256] The lipid content of the product was analysed by methylating a known amount of product followed by analysis on GCMS. Other fatty acids and components were analysed by TMS method on GCMS. Lipid analysis and other fatty acids and components results are given in Tables 9 and 10 below.

Table 9. µmol% per total µmol of FAME

Sample	C4:0	C16:0	C18:0	C18:1	C18:1iso	C18:2	C18:3N3	% oil/DW
Lypozyme batch 4	5.64	19.66	4.04	10.31	0.99	54.18	5.19	41.6

"C18:1iso" includes C18:1Δ9 and CC18:1Δ11

Table 10. Fatty acids and components in minor percentages

	Lypozyme Batch 4 (relative peak intensity)
Glycerides/Free fatty acids	
1,3-Diisobutyrin	3%
1-Mono-isobutyrin	2%
1-Monolinolein	2%
1-Monopalmitin	1%
2-linoleoylglycerol	0%
2-Mono-isobutyrin	0%
2-Palmitoylglycerol	0%
9,12-Octadecadienoic acid (Z,Z)	0%
Glycerol monostearate	1%
Octadecanoic acid	0%
Oleic Acid, (Z)	0%
Palmitic Acid	0%
Sugars/Sugar derivatives	
D-Chiro-Inositol	0%
Disaccharide 1	10%
Disaccharide 2	6 %
Galactinol	19%
Glucopyranose	8%
Glycerol	1%
Glycerol-3-phosphate	1%
Inositol, myo	0%
O-Methyl-inositol	2%
Raffinose	2%

Saccharic acid	0%
Sucrose	0%
Organic acids	
Citric acid	2%
Malic acid	19%
Others	
2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)-4,5-dihydrooxazole Results	1%
Oxazole, 4,5-dihydro-2-pentadecyl	1%
Unknown 1	15%
Unknown 2	0%
Unknown 3	0%

f) Condition optimization

[257] Studies were performed to determine the optimum mol ratio of lecithin:tributyrin, the optimum concentration of lipase and the optimum duration of reaction. As shown in Figure 1A, 1:3 and 1:5 mol ratio gave similar C4 incorporation at 10% enzyme concentration at 65°C. When the enzyme was doubled, C4 incorporation increased. While a lecithin:tributyrin ratio of 1:5 appeared the most efficient, a ratio of 1:3 ratio resulted in good yields and at much lower cost. C4 incorporation increased significantly up to 48 hrs, then tapered off (Figure 1B). In view of these results, a 1:3 mol ratio with 20% enzyme concentration and a 48hr reaction time was deemed optimal.

Example 2 - Studies of emulsion products

a) Sample preparation

Water-oil emulsion (3% w/w fat, 0.5% w/w sample):

[258] A C4-phospholipid sample obtained as described in Example 1a or soy lecithin (50 mg) was added to RO water (9.7 g), vortexed and allowed to hydrate for 30 min at room temperature. After hydration, safflower oil (0.3 g) was added to the water mixture and homogenized for 4 min at setting 5 (IKA T-10 homogenizer). For the water-oil control, the same preparation procedure was followed but no C4-phospholipid sample or lecithin was added.

b) Physico-chemical and functional properties assessment

i. Particle size distribution

[259] The particle size distribution of the samples (cow's milk and water-oil emulsions) was measured using laser diffraction with a Malvern Mastersizer 3000. The samples were diluted in the instrument until the laser obstruction value was ~5%. An absorption coefficient of 0.001 and refractive indexes of 1.46 and 1.33 were used for the emulsion droplets and water, respectively. The volume weighted diameter D[4,3] was recorded. Triplicate measurements were performed for each sample.

[260] Droplet size results are shown in Figure 2, and in Table 11 (Lipid droplet size D[4,3] in unhomogenized and homogenized full cream milk as well as water-oil emulsion with and without 0.5% (w/w) C4-phospholipid (C4-PL, lipase batch 1) or soy lecithin) below.

Table 11

Sample	**D[4,3] µm**	**Std dev**
Unhomogenized full cream milk	4.15	± 0.02
Homogenized full cream milk	0.58	± 0.00
Water + oil	187.53	± 76.09
Water + oil + soy lecithin	219.94	± 39.57
Water + oil + C4-PL (lipase batch 1)	6.85	± 2.35

[261] The C4-phospholipid-containing emulsion was found to have a similar droplet size to unhomogenised whole dairy milk, and smaller than the lecithin-containing control. Lipid droplet size is an important parameter in dairy and food emulsion applications due to its critical impact on the physical stability (e.g., emulsion stability), mouthfeel perception and flavour release. In general, a smaller particle size is preferable in dairy emulsion applications (particularly liquid and semi-solid foods, e.g., yoghurt, milk) as it can enhance the emulsion stability and improve the mouthfeel (e.g., creaminess, smoothness vs. graininess) and taste perception. The smaller lipid droplet size in the emulsion system containing C4-phospholipid compared to the system without C4-phospholipid or the system added with lecithin observed here suggests that C4-phospholipid is a promising agent for emulsifying and stabilising the system.

[262] The particle size of droplets produced using C4-PL from the lypozyme batch 1 preparation was also assessed in the same manner and compared to the C4-PL sample obtained as described in Example 1a (lipase batch 1). As shown in Figure 2B, C4-PL produced with lipase batch 1 and lypozyme enzyme batch 1 generated fat droplets with similar D[4,3] values and size distribution, with the D[4,3] value of the water + oil + C4-PL (lypozyme batch 1) was 11.60 µm (compared to 6.85 µm for water + oil + C4-PL (lipase batch 1); see Table 11. The particle size of the fat droplets in both C4-PL batches are significantly smaller than the lecithin containing control sample. The smaller particle size of the C4-PL containing samples is likely one of the key factors contributing to the enhanced emulsion stability of the samples containing C4-PL compared to the control and lecithin containing samples (described below).

ii. Microstructure Characterization by Confocal Laser Scanning Microscopy (CLSM)

[263] The samples (cow's milk as control and water-oil emulsions) were stained with Nile Red and Rh-DOPE to visualize the lipid droplet and phospholipid membrane, respectively. For observation of the lipid droplet, 1 mL of sample was mixed with 10 µL of Nile Red (1 mg/ml) and stained for 60 min at room temperature. For observation of the phospholipid membrane, 40 µL of Rh-DOPE (1 mg/ml) was pipetted into a dark vial and chloroform was evaporated. Sample (1 mL) was then added into the vial, mixed and stained for 1 hour at room temperature. To reduce the mobility of the lipid

droplets during CLSM observation, the stained sample was mixed with a fourfold volume of 5 mg/mL agarose solution (5 µL stained sample: 20 µL agarose solution) before deposition onto a microscope slide and coverage with a 0.17 mm thick coverslip. The samples were then observed using an inverted confocal laser scanning microscope Leica SP5 (Leica Microsystems). The excitation/emission wavelengths were set at 480 nm/ 500-530 mm, and 561 nm/565-615 nm for Nile Red and Rh-DOPE, respectively. Several images were taken for each sample and the typical images were presented.

[264] The microstructure of the C4-phospholipid containing product is shown in Figure 3A and 3B. The lipid droplets contain a lipid globule core mainly comprised of triacyl glycerol (TAG) (Figure 3A) surrounded by an outer membrane rich in phospholipid (Figure 3B). This structure is similar to that of mammalian milk fat (e.g., unhomogenised cow's milk, Figure 3C), which also contains a TAG globule core and a phospholipid membrane.

[265] The milk fat globule membrane (MFGM) of cow and other mammals (human, sheep, goat, buffalo, etc.) has been well accepted to contain three layers, mainly comprised of phospholipids, sphingomyelin, cholesterol and enzymes/proteins/peptides (see Figure 3D for a schematic diagram of the mammalian MFGM). Several of these components have bioactivities and bio-functional modulation/regulation roles, and are reported to play an important role in the development of infant brain and gut immune system. For example, xanthine oxidoreductase (XO/XDH), a key enzyme found in MFGM, has long been recognized as an important enzyme in the host defence system and in innate immunity and inflammatory responses (Harrison, 2006; Zou et al., 2022). XO/XDH can also impact the flavour and chemical stability of cheese and dairy products (Harrison, 2006). Butyrophilin (BTN) and adipophilin (ADPH) are involved in the regulation of fat metabolism, immune response and body physiological functions (Chong et al., 2011; Eichinger et al., 2021; Matareed et al., 2023).

[266] In summary, in the above experiments a basic model of the fat globules resembling those in mammalian milk, with a TAG core surrounded by a membrane rich in phospholipid, has been established using a C4-phospholipid emulsion system. It is noted that with the current CLSM technique and stains used, the specific details in the structure of the membrane of the C4-phospholipid emulsion samples (e.g., number of layers, arrangement of components within the membrane, etc.) have not been determined, which warrants further investigation.

iii. Rheological assessment of emulsions

[267] Rheological behavior of the samples (cow's milk and water-oil emulsions) was assessed using an Anton Paar MCR 302 rheometer with a double gap cup and bob geometry. Samples were first equilibrated to 20°C for 5 min and flow curves were obtained from stepped shear rate ramp between 0.1 s^{-1} and 1000 s^{-1} at 20°C. The shear rate was increased logarithmically in order to collect 21 data points during shear rate ramp. The viscosity was plotted against shear rate.

[268] The flow curve of the samples is shown in Figure 4. While the water-oil sample displayed a shear thinning behavior, the viscosity of the emulsion containing C4-phospholipid or soy lecithin remained relatively stable as the shear rate increased. This suggests that the addition of lecithin or C4-phospholipid to a water-oil emulsion might have a stabilizing effect.

[269] At shear rate > 100 1/s, no significant differences in viscosity were observed between the three different water-oil emulsions. The viscosity of both the unhomogenized and homogenized full cream milk samples was higher than that of the water-oil emulsions, likely due to the presence of protein in cow's milk that is absent in the water-oil emulsions.

iv. Rheological assessment of C4-skim milk

[270] C4-PL from the lypozyme batch 1 or soy lecithin (50 mg) was added to skim milk (9.7 g), vortexed and allowed to hydrate for 30 min at room temperature. After hydration, 0.3 g of safflower oil was added to the skim milk mixture and homogenized for 4 min at setting 5 (IKA T-10 homogenizer). A skim milk-oil control was prepared using the same procedure but without addition of C4-PL sample or lecithin. Rheological behavior of the samples was then assessed as described above.

[271] As shown in Figure 5, addition of the C4-PL to a skim milk-oil emulsion was found to increase the viscosity of the system. Changes in viscosity were not observed when soy lecithin was added to the skim milk-oil system. These findings suggest that C4-PL might have the ability to interact with milk proteins.

v. Foaming Capability and Foam Stability (Lypozyme batch 1 and 2 – Example 1b)

[272] The impact of C4-phospholipid on the foamability of skim milk was assessed and compared to that of full cream milk, skim milk and skim milk containing lecithin. Full cream milk as well as skim milk at 10°C with and without the addition of 0.5% or 1% (w/w) of C4-PL or (1%) lecithin were frothed with a handheld milk frother for 2 min. The total volume of sample and volume of foam produced was determined and recorded over a period of 60 min (reading at time 0, 5, 10, 15, 20, 30, 60 min).

[273] Foam capacity was defined as the increase (expressed as %) of the volume of the solution after whipping in comparison to the volume of the solution prior to whipping, and determined as follows:

$(V_2-V_1)/V_1 \times 100$, where V_2 (ml) is the volume of solution after frothing and V_1 (ml) is the volume of the solution before frothing.

[274] The foam stability was evaluated as the remaining foam volume after 30 and 60 min and expressed as percentage of the foam volume (ml) immediately after frothing. Results are given in Table 12 below.

Table 12. Foam capacity and stability of milks with or without C4-PL or soy lecithin.

	Sample	**Foam capacity (%)**	**Foam stability (30 min, %)**	**Foam stability (60 min, %)**
	Full cream milk	0	n.a	n.a
	Skim milk	275	100	40
	Soy lecithin, 1% + Skim milk	25	88	83

Lypozyme batch #1	C4-PL, 0.5% + skim milk	50	83	77
	C4-PL, 1% + skim milk	50	83	80
Lypozyme batch #2	C4-PL, 0.5% + skim milk	210	97	81
	C4-PL, 1% + skim milk	150	100	90

[275] Among all samples investigated, skim milk had the highest foam capability (275%) while full cream milk had the lowest foam capability (0%). The addition of C4-phospholipid was found to reduce the foamability of skim milk at 10° C. However, the reduction in foamability with C4-phospholipid was less significant than that observed when lecithin (1%) was added to skim milk. The two lypozyme C4-phospholipid batches were found to differ in their impact on skim milk foamability. Batch 1, with an incorporation of C4 in the final product about 4 times higher than batch 2, had a stronger inhibitory effect on skim milk foaming. This finding suggests that the addition of C4-phospholipid may help reduce foaming in beverage applications.

[276] In contrast to foam capability, skim milk alone had the lowest foam stability, with only 40% of foam remaining 60 min after frothing. On the other hand, one hour after being frothed samples containing C4-phospholipid retained 77-90% of their initial foam volume, while 83% of the foam generated from the skim milk sample containing soy lecithin remained. An increased inclusion rate of C4-phospholipid (1%) improved foam stability compared to when 0.5% C4-phospholipid was added, with a more enhanced effect observed with the lypozyme batch #2 sample. These results suggest that C4-phospholipid might be used as a foam stabilising agent rather than a foam producing agent. The foam stabilizing property of C4-phospholipid could help to decelerate the foam decaying, and hence, increase the stability of foam-containing products during transportation and storage.

Example 3 - Emulsion stability

Experiment 1. Emulsion Stability Assessment

a) Sample preparation.

[277] A C4-PL sample obtained as described above (using lypozyme enzyme, batch 1) or soy lecithin (50 mg or 100 mg) was added to RO water (9.7 g), vortexed and allowed to hydrate for 30 min at room temperature. After hydration, 0.3 g of an annatto oil extract (Vinaco) diluted 4 times in safflower oil was added to the water mixture and homogenized for 4 min at setting 5 (IKA T-10 homogenizer). A water-oil control was prepared using the same procedure but without addition of C4-phospholipid sample or lecithin.

b. Stability assessment

[278] Immediately after emulsification, the samples (2 ml) were pipetted into serological pipettes and the pipettes were sealed with wax. The volume of oil forming at the surface of the emulsion was recorded over time. At least two measurements were performed for each sample. Volume of oil observed at the surface of the emulsion over time is shown in Table 13.

Table 13. Volume of oil observed at the surface of the emulsion over time.

Sample	Volume of oil (ml)								
	5 min	10 min	20 min	30 min	60 min	90 min	120 min	4 hrs	24 hrs
Water + oil	0.01	0.02		0.03	0.04	0.04	0.04		0.08
Water + oil + lecithin (0.5%)		0.02		0.02	0.04	0.04	0.04	0.06	0.08
Water + oil + lecithin (0.5%)		0.02		0.02	0.04	0.04	0.04	0.045	0.08
Water + oil + C4PL (0.5%)			0.01	0.015	0.03	0.04	0.04	0.06	0.07
Water + oil + C4PL (1%)				0.01	0.02	0.04	0.04	0.04	0.06

[279] During the first 60 min following emulsification, the C4-PL-containing emulsions showed less oil on the surface compared to the control or lecithin containing emulsion, indicating the C4-PL containing samples were more stable than the control or lecithin-containing-emulsion samples. Increasing the concentration of C4-PL (0.5% to 1%) further slowed down the destabilization of the emulsions. These results suggest that short chain phospholipids could help stabilize a food system and slow down the oiling-off or separation of oil-in-water emulsions.

Experiment 2. Lace/Dentelle batter stability (cheese dentelle application)

a) Materials and Methods

[280] The batter emulsion was prepared by mixing 40 g water, 60 g safflower oil, 10 g wheat flour, 0.5 g salt and 1g C4-PL (Batch 4) in a bowl using a whisk until the ingredients were thoroughly combined (approx. 30 s). A control sample was also prepared using the formulation above without C4-PL addition. The two batters were then left for resting for 15 min and the physical appearance of the two batters was observed.

b. Results

[281] An obvious phase separation was observed in the batter emulsion without C4-PL inclusion, while this phase separation was not visible in the sample added with C4-PL (Figure 6), suggesting a better stability of the sample was obtained when C4-PL was added. This result is consistent with the findings obtained in the experiment 1.

Example 4 – Sensory evaluation in food matrices

a) Development of sensory lexicons describing the aroma, taste and mouthfeel of commercial plant-based and cow's milks.

[282] Ten commercial unsweetened oat milk products were purchased from the local supermarket and screened by two scientists to select the four beverages displaying the least plant-based aroma and taste. Refrigerated and UHT skim and full cream cow's milk (Canberra and Devondale brands, respectively) were also purchased from local supermarkets. A focus group (5-6 participants, both male and female with age ranging from 23-65) were served randomly coded oat or cow's milk samples and their aroma, taste and mouthfeel was discussed as a group. Terms used to describe the milks were compiled and categorized.

[283] Terms describing the aroma, taste and mouthfeel of oat and cow's milks were recorded and summarized. Several terms used to describe the oat milk beverages were also reported for cow's milk. The main terms used to describe the oat products were related to sweetness and cereal, while the mouthfeel was primarily described as being sandy, grainy, gritty, chalky and powdery. On the other hand, both types of cow's milks were primarily described as having a dairy and buttery/creamy aroma, and a sweet, dairy, vanilla taste. The mouthfeel of skim milk was characterized primarily as being watery and thin while full cream milk was described as being creamy with a fatty mouthfeel. Ultra-high temperature (UHT) processing uses higher temperature than pasteurisation treatment. Increased processing temperatures promote the reaction of amino acids with lactose leading to the formation of Maillard reaction products. As a result, the UHT milk products have a stronger cooked and caramel flavour than pasteurized milks.

[284] Accordingly, a lexicon to describe the aroma, flavor and mouthfeel of dairy and plant-based dairy beverages was developed. The differences in the lexicons between the dairy and plant based dairy products reflect their differences in the sensorial perception and the physicochemical properties (including composition, rheological properties, etc.) as well as the ingredients used in the processing.

b) Dairy and food products

[285] C4-PL Lypozyme batch 1 and C4-MAG obtained as described in Example 1 were incorporated into the following food bases in order to assess the sensory impact:

i. Margarine (Nuttelex Original)
ii. Chocolate
    - Hazelnut based (Vego milk chocolate)
    - Oat based (Lindt, Vegan Smooth milk chocolate)
    - Oat based (Whittakers chocolate)
    - White chocolate (Sweet William)
iii. Australian skim milk (Home brand, Woolworths).

[286] To prepare the margarine samples, soy lecithin or C4-PL was rehydrated in a minimal volume of water with stirring until dissolved, and then combined with Nuttelex Original™ at a 0.5% inclusion rate (w/w). The resulting combination was beaten until combined. A negative control minus the addition of lecithin or sample was included.

[287] To prepare the chocolate samples, C4-PL was homogenized with hot cocoa butter at 40°C for 40 seconds using the IKA T10 Ultra-Turrax (speed 5). C4-PL was added at a rate that achieved 1% concentration (w/w) in the final product. 5% (w/w) of cocoa butter was added to the samples,

which were then tempered to 55°C, cooled to 29°C, before adding 25% (w/w) of un-melted chocolate. The samples were then heated to 32°C and allowed to set on baking paper sheets at room temperature prior to tasting.

[288] To prepare the skim milk samples, soy lecithin and C4-PL were rehydrated in a minimal volume of water with stirring until dissolved. C4-MAG, soy lecithin or C4-PL were then combined with Skim Milk at a 0.5% inclusion rate (w/w) and homogenized for 40 seconds using the IKA T10 Ultra-Turrax (speed 5). An equal volume of the C4-PL in milk and the MAG in milk was combined to produce a C4-PL + MAG sample. A negative control consisting of skim milk alone was also included.

Sensory evaluation

[289] Up to 5 experienced participants (three female, two male, aging from 27-40) were asked to sniff and taste the sample sets in random order. The participants were requested to evaluate the aroma and taste for the major flavors present as well as any dairy-like mouthfeel, compared to the negative control. Participants were also asked to identify the addition rate and matrix where the biggest impact was achieved. Participants then discussed the results in a group format.

Results

[290] When added to dairy milks, the C4-PL and C4-MAG products delivered a dairy note and/or cheesy flavour. This flavour profile of the C4-PL product was found to be distinct from that of traditional, non-modified soy lecithin which lacks any dairy notes. Additionally, in some preliminary applications in food products such as milk, margarine and chocolate, an altered mouthfeel, perceived melting profile, or creaminess was observed with the addition of C4-PL and/or C4-MAG, which mimicked the mouthfeel and sensory experience of a dairy-based product. Accordingly, C4-PL and C4-MAG deliver a cheesy flavour and have the potential to improve mouthfeel and texture in plant-based dairy products.

d. C4-PL in confectionary chocolate (white chocolate) application

[291] Two set of samples of white chocolate were prepared according to the Table 14 below. An amount of 6 g icing sugar was blended and 7.5 g (75% of the total amount) of cocoa butter were melted at 40 °C. The blended sugar was then sieved gradually into the melted butter and mixed for 1 min. Glutinous rice flour (4 g) was then added into set 2 samples. C4-PL was added into the mixture at the inclusion of 1.25-15% as indicated in the Table 14, followed by the addition of 2.5 g of unmelted cocoa butter for tempering purpose. The samples were then strained and moulded before cooling down.

[292] Samples added with C4-PL had a more overall enhanced flavour (sample 2 and sample 4), with a slight cheesy note recorded (sample 4) and a smoother structure and closer flavour to milk chocolate (sample 2).

Table 14. Composition of chocolate samples with and without the addition of C4-PL

Set 1:	Ingredients	Comments
Sample 1.	• 10g cocoa butter • 6g icing sugar	Sweet, granular structure, Fatty but not mouth coating

Sample 2.	• 10g cocoa butter • 6g icing sugar • 0.25g C4-PL (Batch 4) (~ 1.5% inclusion)	Closer to real white chocolate, more lingering Smoother texture
Set 2:		
Sample 3	• 10g cocoa butter • 6g icing sugar • 4g Glutinous rice flour	No cheesy note noted. Whiter colour than set 1.
Sample 4.	• 10g cocoa butter • 6g icing sugar • 4g glutinous rice flour • 0.25g C4-PL (Batch 4) (~ 1.25% inclusion)	Slightly cheesy hint, which might not be as pleasant in Chocolate, good mouth coating effect

e. C4PL in bakery applications

Shortbread cookies

[293] 2 g C4-PL (lypozyme Batch 4) was rehydrated in 10 g water before adding into a mixture of 50 g (melted) cocoa butter and 20 g icing sugar. Flour was then added gradually into the mixture and mixed for 2 min or until smooth. The dough was then divided into 4 portions. Each portion was rolled into 5 cm long logs, wrapped in cling film and placed in the fridge to set for at least 2 hours. After that, the cling film was removed and the logs were sliced into 5 mm discs, which were then placed on the baking tray and baked in the oven for approx. 8 min at 160 °C. The cookies were then removed from the oven, and placed on a wire rack to cool. A control sample was also prepared using the procedure as described here but without the addition of C4-PL.

[294] Samples added with C4-PL were commented as having significant differences compared to the control sample (without C4-PL). These differences included the dairy like-aroma, the fatty mouth coating effect and creamy texture in the samples with C4-PL inclusion.

Cheese laces

[295] The lace batter was prepared by mixing 40 g water, 60 g safflower oil, 10 g wheat flour, 0.5 g salt and 1g C4-PL (Batch 4) in a bowl using a whisk until the ingredients were thoroughly combined (approx. 30 s). A control sample was also prepared using the formulation above without C4-PL addition. Approx. 5 to 7 g of the two batters were then poured onto a non-stick pan pre-set at medium heat, with a temperature of 160 to 180 °C and cooked for approx. 1-2 min until the laces become golden. The laces were then flipped and cooked on the other side for another 30s.

[296] Lace sample with C4-PL inclusion had a cheesy-snack like note with a significantly different structure compared to the control sample (Figure 7). C4-PL containing sample had a denser and finer web net structure and an enhanced elasticity, likely to be related with the higher emulsion stability that was observed in the lace batter. This finer and denser web net structure could help trap and retain the flavour/volatile compounds within the network and hence reduce the flavour/aroma loss during the cooking/heating process. As such, C4-PL is a promising ingredient in bakery applications.

f. C4-PL in beverage applications:

C4-PL in water

[297] C4-PL was added into water at the inclusion rate of 1%. The mixture was then mixed using a homogenizer IKA T10 Ultra-Turrax for 30 s at speed 6. The control sample was water. Eight participants were asked to sniff and taste both samples and describe the flavour they perceived.

[298] The sample added with C4-PL was commented as having a cheesy and milky note compared to the control sample. There were also some comments on the metallic and whey-like flavour in C4-PL added samples.

C4-PL in skim milk

[299] C4-PL was added into skim milk (0.3% fat) at the inclusion rate of 1%. The mixture was then mixed using a homogenizer IKA T10 Ultra-Turrax for 30 s at speed 6. The control sample was skim milk only. Eight participants were asked to sniff and taste both samples and describe the flavour they perceived.

[300] The skim milk samples with C4-PL were observed to have a better mouthfeel with more creaminess and cheesy notes than skim milk alone.

Example 5. Additional C4-PL preparations

[301] Additional C4-PL preparations were produced as described below.

A. Preparation of batches 6.2, 9, 10, 11.2 and 13.2

[302] Materials used in the production of the C4-PL included Soy lecithin (de-oiled powder) (The Melbourne Food Ingredient Depot), Tributyrin (Penta International Corporation), Lipozyme® TL IM (Novozymes), Acetone (Sigma-Aldrich) and n-Hexane (Merck).

[303] Ingredients including tributyrin and soy lecithin were mixed at 3:1 mol ratio. 10% Hexane (Hexane:substrate w/w%) and 20% lypozyme (Lypozyme: total reaction mass w/w%) were combined in BPA free plastic pouches (Proline; 30 x 35 cm). The pouches were vacuumed, sealed and incubated at 45°C in a water bath (Thermo Scientific Precision SWB27) with agitation at 75rpm for 72 hrs.

Extraction/Purification - Batch 6.2

[304] The reaction mixture was scraped out of the pouches and the enzyme was removed by filtration. The filtrate was then left gravimetrically separate to oil layer and enriched phospholipids. The top oil layer was decanted and stored in -20°C. The phospholipid layer was diluted in 1:2 water (pellet:water w/w). Diluted phospholipid mixture was precipitated by four acetone washes at room temperature to obtain a pellet enriched in short chain phospholipids. The oil layer was subsequently washed with acetone 1:1.22 ratio and 3 further washes with 1:2 ratio using ice cold acetone (oil layer: acetone w/w). Acetone insoluble fraction from both phospholipid fraction and oil layer were collected by filtration. The retentate was resuspended in 1:3 RO water (retentate:water w/w) and stirred using an IKA overhead stirrer until complete solubilization. The mixture was then freeze dried until completely dry.

Extraction/Purification - Batch 9, 10, 11.2 and 13.2

[305] The reaction mixture was scraped out of the pouches and the enzyme was removed by filtration. The filtrate was then precipitated by 1:5 ratio (Reaction mixture: acetone w/w) and subsequent acetone washes (pellet: acetone 1:2.2 w/w) at room temperature to obtain a pellet enriched in short chain phospholipids. Acetone insoluble fraction from both phospholipid fraction and oil layer were collected by filtration. The retentate was resuspended in RO water and stirred using an IKA overhead stirrer until complete solubilization. The mixture was then subjected to the drying process. Batch 9 was freeze dried, batch 10 ,11.2 and 13.2 were oven dried to obtain a fine powder.

Drying process

[306] The mixture was dried following one of the two methods as described below:

[307] Freeze drying process: The mixture was frozen at -80 °C prior to being freeze dried (Labconco bulk tray dryer) for 31 hrs using a drying cycle of increasing temperature (-20°C for 16 hrs, followed by a progressive increase to 60°C over a 15 hrs period). The dried sample was then ground into a fine powder. As the acetone level was high, the product was resolubilized in RO water and freeze dried again using the same drying cycle as described above. The dried sample was grinded and stored in vacuum sealed pouches (Proline; 30 x 35 cm).

[308] Oven vacuum drying process: The mixture was oven vacuum dried at 80°C and 10 mbar using a vacuum oven (Across International). During the drying process, the sample was taken out every 20-30 min to mix and re-spread into thin layers until the sample was fully dried. The sample was then grinded and passed through a sieve to obtain a fine powder. As acetone level was high, the product was resolubilized in water and oven dried again using the same procedure as described above. The final dried product was again grinded and passed through a sieve to obtain a fine powder and stored in vacuum sealed pouches (Proline; 30 x 35 cm).

B. Preparation of batches RPR 002, 004 and BRprep17_EtOH

[309] A hexane-free process using a bioreactor was also used to prepare C4-PL. This method eliminates Hexane from the process which eliminates the safety hazard during batch reaction.

[310] Materials used in this process included Soy lecithin (Shanker), Tributyrin (Penta International Corporation), Lipozyme® TL IM (Novozymes), Acetone (Sigma-Aldrich), Ethanol (Ethanol 95, Redox).

[311] Ingredients including tributyrin, soy lecithin (tributyrin:soy lecithin 2:1 w/w) and RO water (RO water: Soy lecithin 0.04:1 w/w) were combined in 2L Bioreactor (Satorius). The mixture was stirred at 75rpm at 45°C until the temperature equilibrium was reached. Lipozyme was added (Enzyme: total reaction mixture 0.05:1 w/w) and mixing continued at 75rmp for 48hrs at 45 ^{o}C.

[312] The reaction mixture was then subjected to extraction/purification step.

Extraction/Purification (Ethanol/Acetone) - Batches RPR 002 and 004

[313] The reaction mixture was harvested and diluted with addition of 20% Ethanol (Ethanol:Reaction mix w/w%) and enzyme was removed by filtration using a nylone filter bag. The filtrate was then precipitated by several acetone washes at room temperature to obtain a pellet

enriched in short chain phospholipids. The precipitate was resuspended in RO water and stirred using an IKA overhead stirrer until complete solubilization. The mixture was then heated at 105oC for 30min to heat inactivate any enzyme residue. Finally, the samples were subjected to drying to dehydrate and remove any residual Acetone.

Extraction/Purification (water degumming/ethanol) - Batch BRPrep017 EtOH

[314] The reaction mixture was harvested and diluted with addition of 10% water (Water: Reaction mix w/w %) and enzyme was removed by filtration using a nylone filter bag. Filtrate was then left to gravimetrically separate to an oil layer and an enriched phospholipids layer (gum). The oil was poured off and the gum was washed 3 times with 25% Ethanol (Ethanol:Reaction mixture w/w%) and freeze dried.

Enzyme inactivation

[315] A further enzyme inactivation step was included to inactivate any residual enzyme. Dried product was diluted in water at 1:1, 1:2 and 1:3 ratio (product:water w/w) and heated to 105 and 135°C for 5min and 30min. Enzyme activity was measured using commercially available Lipase detection Kit II (ab102525, Abcam). Enzyme was fully inactivated at 105 °C for 30 min and 135°C for 30 min, while residual activity was detected at both treatments done for 5min duration. Product treated at 135 °C was oxidized, causing a colour change of the product. Samples diluted at 1:1 and1:2 also showed discoloration. Therefore 1:3 dilution at 105 °C for 30min treatment was selected as the method of enzyme inactivation for successive baches. The product was then freeze-dried to remove the water after the heat treatment.

C. Analysis

The compositions were analysed to determine the content of the samples. Lipid and fatty acid profiles were assessed using methods essentially as described in Example 1. Results are provided below.

Table 15. Proximate composition of C4-PL batches 7, 9, 10, 11.2, 13.2 produced using the pouch system. "OD" = oven dried; "FD" = freeze dried.

Preparation	Moisture (g/100g)	Fat (g/100g)	Protein (g/100 g)	Ash (g/100g)	Carbohydrates (g/100g)
De-oiled soya lecithin	2.6	58.5	6.6	7.6	25
9 FD	3.3	52.5	7.4	3.3	34
10 OD	2.4	55.2	7.3	11	24
11.2 OD	1.9	55	7.3	13.9	22
13.2 OD	2.1	55.3	7.1	12.3	23

"OD" = oven dried; "FD" = freeze dried.

Table 16. Fatty acid profile of C4-PL batches.

Sample	***µmol % per total µmol of FAME***							% oil/DW[1]
	C4:0	C16:0	C18:0	C18:1	C18:1 iso	C18:2	C18:3	
6.2 FD	3.1	20	4.1	10.2		55.8	5.8	41.7
9 FD	6.8	19.1	3.8	9.8	1.0	53.8	5.7	45.30
10 OD	6.8	20	3.9	9.7	1.0	53.1	5.7	52.63
11.2 OD	5.6	20.4	4.0	9.8	0.9	53.6	5.8	50.9

13.2 OD	5.6	20.6	4.1	9.9	0.9	53.2	5.8	54.7
RPR002	16.0	18.0	2.4	15.4	1.2	43.8	3.2	49.7
RPR004	12.7	19.2	2.6	16.1	1.4	44.7	3.2	53.8
RPR17-EtOH	11.54	21.50	3.07	15.96	1.49	43.32	3.12	57.21

"C18:1iso" includes C18:1Δ9 and CC18:1Δ11

[316] A further analysis of batches 9 and 10 confirmed that there was no C4 free form (butyric acid) in the samples; 100% of the C4 was present in the bound forms (25-27% in monoacylglycerol, and 73-75% was in PL forms). In analysis of batches 11.2 and 13.2, monoacylglycerol was below the level of detection, *i.e.* the bound C4 was essentially all C4-PL.

[317] The phospholipid profiles of batches 9 and 10 were also analysed and are presented in Table 17. As expected, the fat fraction in of the C4-PL preparations consists mainly of phospholipids (>99%). Within the phospholipids profile, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the two major classes (60-62%), followed by other phospholipids (e.g., PI, PA, PG, 26-34%) and lysophospholipids (LPLs) (6-12%). These results are expected and in agreement with the lipid profile of lecithin, wherein PC and PE comprise the most PL fractions.

Table 17. Phospholipid profile of batches 9 and 10.

Sample	**% w/w in total phospholipids**			% total PL/DW	% total fat/DW	% total PL/total fat
	PC+ PE	Other PLs (PI, PA, PG)	LPLs			
Freeze dried B9	60	34	6	45.10%	45.30%	99.56%
Oven dried B10	62	26	12	52.30%	52.63%	99.37%

"PC" = phosphatidylcholine, "PE" = phosphatidylethanolamine, "PI" = phosphatidylinositol, "PA" = phosphatidic acid, "PG" = phosphatidylglycerol (PG), "LPL" = lysophospholipid, "PL" = phospholipid, "Dw"= dry weight of C4-PL.

D. Additional characterization of C4PL

[318] The total fatty acid content and the phospholipid profile of C4-PL was further analysed and compared to soy lecithin.

As shown in Table 18 the total fat content is essentially the same between the two preparation, with the exception of 6.68% of the long chain fatty acids being replaced with C4:0 in the C4-PL preparation compared to soy lecithin.

Table 18. Total fatty acid composition of soya lecithin (prior to interesterification) and C4PL.

Fatty acid composition %		
	Soy lecithin	**C4PL**
Total fat (% oil/Dwt)	**56.45**	**53.92**
of which saturated (%)	26.25	30.68
of which monounsaturated (%)	11.57	10.61
of which polyunsaturated (%)	62.19	58.71

Fatty acid composition		
SCFA (C4:0) (%)	0.00	6.68
MCFA (%)	0.15	0.24
LCFA (%)	99.85	93.08

[319] The relative abundance of PL classes in the C4PL preparation was assessed on a Shimadzu Nexera LC coupled with Sciex 7600 HRMS. Data was acquired on MRM mode. Comparison of PLs between the soy lecithin and C4-PL (Table 19) shows that PL species are the same between the two types of lecithin, and include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), and lyso phospholipids (LPC, LPE, LPI, LPS and LPA). The major modification between the two types of lecithin is where C4:0 containing PL species are not detected in the soya lecithin and they are present in C4-PL. Exchange of long chain fatty acids to C4:0 occurs mainly in PC and PE. PC undergoes hydrolysis and hydrolysed products are removed in the extraction process. PE and PS also undergo hydrolysis but are retained during the extraction process, and therefore the LPE and LPS levels are increased in C4PL compared with the soya lecithin.

Table 19.

Phospholipid composition relative abundance			
PL class	**PL Type**	**Soya Lecithin**	**C4PL**
PC	PC	69.23%	35.95%
	LPC	3.94%	0.39%
	C4-PC	0.00%	6.85%
PE	PE	19.09%	21.61%
	LPE	0.08%	11.44%
	C4-PE	0.00%	7.27%
PS	PS	6.42%	7.34%
	LPS	0.06%	6.32%
	C4-PS	0.00%	0.03%
PI	PI	0.57%	1.37%
	LPI	0.11%	0.04%
	C4-PI	0.00%	0.97%
PA	PA	0.48%	0.29%
	LPA	0.02%	0.08%
	C4-PA	0.00%	0.04%

Example 6 – Stability of C4-PL preparations

[320] C4-PL was assessed for its resistance under different processing conditions commonly used in food industry, including shear, pH and UHT treatments

Sample preparation

[321] *Ultra High Temperature (UHT) treatments:* C4-PL batch 11.2 (2 g) was reconstituted in room temperature water (8 g) and heated up to 140°C for 2 sec before being cooled down to room temperature and freeze dried using an Anton Paar rheometer equipped with a pressurised starch cell. The UHT treated sample was freeze dried before being subjected to fatty acid analysis. The heat-treated samples were then fractionated by TLC and the phospholipid bands were analyzed on GCFID for their fatty acid content.

[322] *pH treatment:* C4-PL batch 11.2 was added to RO water at a 3% inclusion rate and let to hydrate at room temperature for 15 min. The mixture was then homogenized using a T-10 Ultra Turrax homogenizer at setting 5 for 2 min. An aliquot of the solution was adjusted to pH 3.0 using lactic acid while another aliquot was adjusted to pH 8.0 using 5N NaOH. Both aliquots were then freeze dried before being assessed by TLC fractionation. The phospholipid bands were subsequently analyzed for their fatty acid content by FAME.

[323] *Shear treatment:* C4-PL batch 11.2 was added to RO water at a 1% inclusion rate and mixed for 30 sec at room temperature in a Thermomix at setting 6 (3000 rpm). The solution was then warmed up for 4 min until it reached 90°C and mixed at this temperature for 2 min at setting 6. The solution was cooled down before being freeze dried. The treated sample were then assessed by TLC fractionation and the phospholipid bands were analyzed for their fatty acid content by FAME.

[324] An untreated solution of C4-PL batch 11.2 (1%) in water was used as control.

Results

[325] Table 20 provides a summary of the results. No significant changes were observed in the fatty acid composition after any of the applied treatments. These results suggest that the fatty acid composition of C4-PL product is stable under acidic, basic conditions as well as under food processing conditions involving shearing and heat.

Table 20. Average fatty acids in C4-PL after UHT, pH and shear treatments.

Treatment	mmol% per total μmol FAME						
	C4:0	C16:0	C18:0	C18:1	C18:1iso	C18:2	C18:3N3
Control	6.92	20.02	4.12	10.00	1.05	52.94	4.95
C4-PL at pH 3.0	7.60	19.47	4.03	10.29	1.07	52.69	4.84
C4-PL at pH 8.0	6.78	20.09	4.13	10.09	1.05	52.90	4.96
After shear treatment	6.88	20.31	4.15	10.03	1.06	52.67	4.90
After UHT treatment	6.80	19.79	4.02	10.17	1.04	53.12	5.06
Average	7.00	19.94	4.09	10.12	1.05	52.86	4.94
Std	0.34	0.32	0.06	0.12	0.01	0.18	0.08

"C18:1iso" includes C18:1Δ9 and CC18:1Δ11

Example 7 – Sensory evaluation in food matrices (2)

[326] Various preparations of C4-PL were incorporated into different foods and a sensory evaluation performed.

A. Coffee creamer application:

[327] The use of C4-PL to reduce the palm oil content of a liquid coffee creamer application was investigated by preparing creamers containing various amounts of palm oil (4-8%) and C4-PL Lypozyme batch 11.2 (0-3%). Samples were prepared by combining sugar, tricalcium phosphate, sodium caseinate, carrageenan, trisodium citrate, C4-PL and salt with 70°C water and homogenizing with a Silverson mixer for 2 min at 3500 rpm. Meanwhile, the palm oil was slowly melted on a hot plate before addition of sodium stearoyl lactylate and glyceryl monostearate. The water and fat mixtures were combined with the Silverson for 1 min at 3500 rpm before being passed through a 2-stage PANDA homogenizer (GEA; 1st stage = 350 bar, 2nd stage = 40 bar). After being refrigerated for at least 48 hrs, the coffee creamer (7%) was added to vanilla extract (2%) and instant coffee (1% solubilized in 80°C water).

[328] Sensory evaluations were performed to assess the differences between a control formulation (8% palm oil, 0% C4-PL), reduced palm oil formulation (4-7% palm oil, 0% C4-PL) and reduced palm oil formulation with the addition of C4-PL (4-7% palm oil with 1-3% C4-PL). Panelists (6-8), both males and females with ages ranging from 25 to 60 years old, were asked to evaluate the difference between the samples on the aroma, overall taste and texture. Sample evaluation was performed under red light to avoid any visual bias.

Results

[329] The reduction of palm oil generates a coffee creamer that is less opaque, while addition of C4-Pl produces a creamer that has a beige color (data not shown). Replacing palm oil with C4-PL in the creamer produced a coffee that was perceived as more viscous, less intense in coffee flavor and having less acidic notes compared to the reference (reduced palm oil creamer without C4-PL). Among all the formulations containing varying amounts of palm oil (4-8%, w/w) and C4-PL (0-3%), the creamer containing 4% palm oil and 3% C4-PL was found to generate the coffee that more closely resembled the texture of the coffee produced with the original coffee creamer formulation (8% palm oil). This demonstrates the utility of C4-PL in coffee creamers, partially or fully replacing palm oil.

B. Processed cheese application:

[330] Three processed cheese samples with different C4-PL inclusion rates (0 to 2%), were investigated with final proximate composition presented in Table 21. Processed cheese formulated without C4-PL was prepared as control.

[331] The processed cheese samples were prepared by chopping fresh cheddar cheese curds (23%), 24 months aged cheddar cheese (9%), Havarti (5%) and low fat cheese (5%) into a Thermomix (speed 4, 3 min). Skim milk powder (7.6%), emulsifying salts (1.7%), lactic acid (0.7%), C4-PL batch RPR002 (1-2%) and water (46%) were added to the chopped cheese and mixed for 5 min at speed 5. The mixture was then further mixed (speed 6) at 85°C for 5 min, then 90°C for 7

min before being cooled to 85°C for 2 min and poured in an aluminum mold. Processed cheese formulated without C4-PL was prepared as control.

Table 21. Proximate composition of Processed cheese.

	Control	1% C4-PL	2% C4-PL
Protein (g/100g)	13.28	12.99	12.72
Fat (g/100g)	11.35	12.02	12.54
Carbohydrate (g/100g)	6.31	6.05	5.74

[332] Sensory evaluations were performed to assess the differences between a control processed cheese produced without C4-PL and formulation containing either 1 or 2% C4-PL. Female panelists (5-6) with ages ranging from 25 to 43 years old, were asked to evaluate the difference between the samples overall taste and texture. Observations made by the participants were discussed in a group format.

Results

[333] Cheese samples produced with C4-PL were slightly darker and less firm than the control formulated without C4-PL. While addition of C4-PL had little if any impact on the creaminess of the cheese, the cheese produced with 1% C4-PL was found to have an increased cheesiness compared to the control. However, at higher inclusion (2%), plastic-y, beany and bitter off flavors were perceived. These findings suggest that at low inclusion rate, C4-PL can improve the cheesiness of a process cheese.

C. Oat milk application:

[334] C4-PL batch RPR004 (0.3, 0.5 and 1.3%) was hydrated in oat milk light (1.5% fat) for 10 min at room temperature before being homogenized using a homogenizer IKA T10 Ultra-Turrax for 2 min at speed 5. Oat milk full (2.8% fat) was used as control for the sensory assessment.

[335] Sensory evaluations were performed to assess the differences between the control oat milk full fat and the oat milk light with C4-PL. Female and male panelists (5) with ages ranging from 35 to 43 years old, were asked to evaluate the difference between the sample aroma, overall taste and texture. Observations made by the participants were discussed in a group format.

Results

[336] Addition of C4-PL at all inclusion rates was found to mask the oat/plant aroma and flavor of the oat beverage as well as increase the creaminess. The beverage containing 1.3% C4-PL was perceived as the most similar in creaminess to the reference full fat oat beverage. However, when C4-PL was added at 0.5% or higher, some plastic-y, bitter off-notes as well as astringency were also perceived.

D. Oat & Soy beverage application

[337] Oat & Soy unflavored beverage packaged in hermetically sealed pouches received from a customer to evaluate the effect of C4-PL. C4-PL batch 6.2 was hydrated in Oat & Soy unflavored beverage at 0.3% or 0.5% incorporation for 10 min at room temperature before being homogenized

using a homogenizer IKA T10 Ultra-Turrax for 1 min at speed 5. The unflavored beverage without C4-PL was used as a control for the sensory assessment.

[338] Sensory evaluations were performed to assess the differences between the control and test. Female and male panellists (7) with ages ranging from 27 to 70 years old, were asked to evaluate the difference between the samples on aroma, overall taste, and texture. Observations made by the participants were discussed.

Results

[339] The addition of C4-PL was found to mask the cereal and beany off-notes and increase the creaminess, viscosity, and fat coating in the unflavoured beverage. Some of the panellists also perceived mild sweetness. Plasticky and other off notes were not noticed with 0.3% dosage, however, it was perceived with 0.5% dosage.

E. Chocolate application:

[340] C4-PL batch 13.2 was used in the chocolate applications. Six different trials of chocolate were performed, with detailed formulation presented in Table 22 below.

Table 22. Detailed formulation of chocolate production using different inclusion rates of C4-PL

	Trial 1 50% milk chocolate	Trial 2 40% milk chocolate	Trial 3 40% PB chocolate (0.4% soy lecithin)	Trial 4 40% PB chocolate (0.4% soy lecithin + 1% C4-PL)	Trial 5 40% PB chocolate (1% C4-PL)	Trial 6 40% PB chocolate (0.4% C4-PL)
Cocoa Nibs	250 g (25%)	100 g (10%)	100 g (10%)	100 g (10%)	100g (10%)	100 g (10%)
Coacoa butter	250 g (25%)	300 g (30%)	300 g (30%)	300 g (30%)	300 g (30%)	300 g (30%)
Full cream milk powder	250 g (25%)	200 g (20%)	0	0	0	0
Oat milk powder	0	0	200 g (20%)	200 g (20%)	200 g (20%)	200 g (20%)
White Granulate sugar	250 g (25%)	400 g (40%)	400g (40%)	400 g (40%)	400 g (40%)	400 g (40%)
Vanilla Beans (Scraped)	2	2	2	2	2	2
Soy lecithin	4g (0.4%)	4 g (0.4%)	0	4 g (0.4%)	0	0
C4-PL	0	0	4 g (0.4%)	10 g (1%)	10 g (1%)	4 g (0.4%)

*PB – plant-based

[341] The ingredients (sugar, milk powder and cocoa nibs), melanger mixing bowl and grinding stone wheels were warmed up in an oven set at 50 °C. Once the melanger mixing bowl and stone wheels were warmed up, they were removed from the oven and placed onto the melanger machine.

[342] Cocoa butter and vanilla beans (scraped from the pods) were added into a pot and slowly melted using the oven or stove top with temperature maintained at 50-60 °C. For trials 1-3, soy

lecithin was added together with cocoa butter and vanilla beans, while for trials 4-6, soy lecithin was added at the 30 hr mark. The mixture of melted cocoa butter and vanilla beans was then poured into the pre-warmed melanger mixing bowl and let mix for 5-10 min. Pre-warmed cocoa nibs were added gradually into the mixture in the melanger bowl, followed by the gradual addition of prewarmed milk powder (for trials 1-2 only) and sugar. Once all ingredients were added in, the mixture was grinded and mixed for a total of 36 h (with lid off for the first 3 h to prevent condensation). Soy lecithin an/or C4-PL were added at the 30 h mark. After 36 h of mixing, the mixture was tempered at 26-29 °C for at least 15 min and piped into the pre-warmed molds, which were then let to set in the fridge for 20-25 min before demolding to get the final chocolate product.

[343] The chocolate product of each trial was sensory evaluated for both aroma and flavour. Eight (8) participants with ages ranging from 25-60 joined the sensory evaluation. The sensory attributes including creaminess, oat and plant-based flavour, chocolate flavour and sweetness of the chocolate samples were assessed and recorded.

Results

[344] The six samples exhibited different aroma and flavour attributes. Samples containing C4-PL (trial #4, 5, and 6) showed an improved chocolate flavour with less evidence of oat milk flavour, compared to sample in trial #3 (no C4-PL added), where the oat milk flavour was dominant, masking the chocolate notes. Among the three chocolates samples with C4-PL added (trials #4, 5 and 6), the sample with 0.4% C4-PL (trial #6) was the most promising, having creamy and chocolate flavour, smooth texture, and a more milky flavour with a full mouth coating effect than the benchmark samples of trials #1 and 2, without evident oat milk flavour and off notes. Besides improved flavour, the trial #6 also showed the improved process with increased fluidity of the mixture during tempering phase. This study demonstrated the emulsifying properties of C4-PL as well as it's ability to impart improved flavour, creaminess and mouthfeel.

Example 8 – Additional sensory evaluation in milks

A. C4-PL in skim milk

[345] The impact of C4-PL on the sensory properties of skim milk was further investigated using a batch of product dried by oven drying (batch 10) and freeze drying (batch 9).

[346] The samples were prepared by hydrating C4-PL (batch 9 and 10; 0.5%) in skim milk for 10 min at room temperature. The milk was then homogenized with an IKA T-10 homogenizer for 2 min at setting 5.

[347] Samples were coded and the Degree of Difference sensory evaluation method was used to determine the difference between the milk containing C4-PL and the control skim milk.

[348] Female and male panelists (6) with ages ranging from 25 to 43 years old, were asked to rank the difference level between the samples and the skim milk reference on a scale of 1-5 (1 being "no difference" and 5 "extreme difference") on the overall appearance, odor, taste and bitterness. Observations made by the participants were recorded and further discussed in a group format. Skim milk was used as a reference.

Results

Table 23. Milk samples ranking means.

	C4-PL Batch 9	C4-PL Batch 10	Hidden Ref Skim Milk
Overall Appearance	1.6	2	1.6
Cream Odour	1	1.4	1
Cheese Odour	1	1.6	1
Rancid Odour	1.2	1.4	1
Cream Flavour	2	2	1.4
Cheese Flavour	1	1.2	1
Rancid Flavour	1.6	1.6	1
Bitterness	1.8	1.6	1
Thickness	2	2.4	1.2

[349] Overall, the addition of C4-PL on skim milk increased the milk's dairy notes as well as created a thicker texture.

B. Different C4-PL incorporation in skim milk

[350] C4-PL batches containing different levels of C4-PL incorporation were added to skim milk and their impact on the sensory properties was assessed.

[351] The samples were prepared by hydrating 0.3% of C4-PL batch 11.2 and RPR004, containing 5.5 and 12.7 µmol% of C4-PL incorporation respectively, for 10 min at room temperature. The milk was then homogenized with an IKA T-10 homogenizer for 2 min at setting 5.

[352] Samples were coded and the Degree of Difference method was used to determine the difference between the samples.

[353] Two samples including (i) skim milk containing 0.3% C4-PL batch RPR004 (that has 12.7 µmol % of C4-PL incorporation), (ii) hidden reference skim milk containing 0.3% C4-PL batch 11.2 (which has 5.5 µmol% of C4-PL incorporation) were compared against the control sample, which was skim milk added with 0.3% C4-PL batch 11.2.

[354] Female and male panelists (6) with ages ranging from 25 to 40 years old, were asked to rank on a scale of 0-5 (0 being "no difference" and 5 "extreme difference") the sample on the overall appearance, odor, taste, bitterness and texture compared to the skim milk control containing C4-PL batch 11.2. Skim milk was also used as an internal control. Observations made by the participants were recorded and further discussed in a group format.

Results

Table 24. Milk samples ranking means.

	C4-PL Batch RPR004	C4-PL Hidden Ref Skim Milk
Overall Odour	0	0.25
Cream Odour	0	0
Cheese Odour	0	0

Off notes Odour	0	0
Overall Flavour	0.8	0.8
Cream Flavour	1.2	0.8
Cheese Flavour	1	0.4
Off notes Flavour	1.2	0.2
Bitterness	0.8	0.4
Overall Texture	1	0.6
Fat coating (mouthfeel)	1	0.6
Thickness	1	0.8

[355] Overall, the difference between the odor of the skim milk and skim milk with C4-PL batch 11.2 and RPR004 was marginal. Milk sample containing C4PL batch RPR004 was perceived as having slightly stronger dairy notes and off flavors (i.e. plasticky and grassy) compared to the skim milk on its own and skim milk with C4-PL batch 11.2. In terms of texture, skim milk with C4-PL batch RPR004 that has the higher C4-PL incorporation rate (12.7% µmol) was found to have a slightly creamier texture and thickness compared to the reference skim milk with C4-PL batch 11.2 that has the lower the C4-PL incorporation rate (5.5% µmol).

Example 9 – Viscosity and creaming of C4PL-containing emulsions

[356] Studies were performed to assess the effect of C4PL on viscosity and creaming in emulsions, compared to unmodified soy lecithin.

A. Methods

Emulsion Preparation

[357] Model emulsions were prepared using C4PL batches or soy lecithin. Safflower oil (Plenty Heart Smart) was used as the oil phase and milk protein was a mix of 80% micellar casein and 20% whey protein (Bulk Nutrients). A series of model emulsions were prepared based on the matrix included in Table 25. The model emulsions were homogenised using the Ultra Turrax T25 homogeniser with a 10 mm dispersing tool at 10000 rpm for 2 minutes. The only exception was batch 6.2 where the emulsions were prepared using the handheld Ultra Turrax with a 5 mm dispersing tool.

Rheology

[358] Rheology measurements of the emulsions were carried out on the Anton Paar MCR502 rheometer, using a 50 mm parallel plate at 0.35 mm gap. Viscosity measurements were done using rate-controlled mode, from 1 s-1 to 1000 s-1 at 20oC. All measurements were repeated at least once to ensure reproducibility.

Creaming

[359] Creaming behaviour of the model emulsions was captured using the stability analyser (Turbiscan Lab, Microtrac). 20 ml of emulsion was filled in a transparent vial. The machine emits laser across the height of the vials and collects data of the transmitted and backscattered signal. A

stronger backscatter intensity indicates that a layer of sediment or cream has formed. Each emulsion was monitored for a period of one hour with a scan every minute.

Table 25. Composition of emulsions.

Emulsifier*	Milk protein**	Oil
0.5%	3.3%	3%
0.5%	0%	3%
0.5%	3.3%	0%
1.0%	3.3%	3%
1.0%	0%	3%
1.0%	3.3%	0%
1.5%	3.3%	3%
1.5%	0%	3%
1.5%	3.3%	0%
0%	3.3%	3%

*Emulsifier includes C4PL or soy lecithin

**milk protein was prepared to 80% casein and 20% whey

B. Results

Viscosity

[360] Comparison was made between emulsions prepared with C4PL batch 9 and soy lecithin. Without any milk protein included, the viscosity of model emulsions prepared with C4PL is lower compared to soy lecithin at 1.5% inclusion rate, while viscosities are insignificantly influenced by the type of emulsifier at 0.5% and 1 (data not shown). This may be the result of the lower solubility of soy lecithin in water, as observed by the strong yellowish colour and significantly increased turbidity when suspended at 1.5% concentration. This results in a higher concentration of suspended particles which increases the viscosity. In contrast, for model emulsions containing milk protein, the viscosity of the model emulsion containing C4PL is higher than their counterparts prepared with soy lecithin as shown in Figure 8. At 1.5% concentration of C4PL, the model emulsion starts to exhibit shear thinning behaviour (data not shown).

[361] It was hypothesised that the interaction between C4PL and milk proteins is stronger compared to the interaction between soy lecithin and milk proteins, resulting in the higher viscosity. C4PL showed a distinct influence on milk proteins and their interactions, compared with soy lecithin. At most inclusion rates studied here, C4PL results in a higher viscosity of the milk protein solution than those with soy lecithin. At the same solid content, a solution/dispersion that is more structural with stronger interactions between elements generally exhibits a higher viscosity. This is because in systems with stronger inter-particle correlations, the dynamics of particles is more profoundly influenced by nearby particles, which poses more resistance when the system is being sheared. The consistently higher viscosity of the protein solutions containing C4PL than those with soy lecithin at all inclusion rates is an indication of the ability of C4PL to more effectively promote the interaction between milk proteins, possibly due to its better solubility/dispersibility in water.

Creaming properties

[362] For the emulsions containing milk protein, both sedimentation and creaming occurs as shown in Figure 9. Without any C4PL added, a layer of foam can be seen after the homogenisation process as reflected by the high initial backscattered intensity at the top of the liquid level. With the addition of C4PL, no notable foam layer can be seen. It is also noted that for the emulsions with added C4PL (both 0.5% and 1.5%), by comparing the evolution of backscattered intensity, the creaming occurs more slowly compared to that without C4PL added. In terms of sedimentation, the addition of C4PL reduces sedimentation, which is reflected by the reduced height of sediment layer with increasing concentration of C4PL. Creaming and sedimentation are two of the factors influencing the long-term stability of food emulsions, with both generally being undesirable. As shown here, C4PL can reduce both, demonstrating its potential benefits in food applications as a stabiliser.

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Claims

1. A lipid comprising phospholipids, wherein the phospholipids comprise phospholipid esterified fatty acids, wherein the phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, optionally wherein:

the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol C4 fatty acids; and/or

the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC) and from about 15% to 35% phosphatidylethanolamine (PE).

2. The lipid according to claim 1, wherein the phospholipids further comprise one, two, three or all of phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), and lysophospholipids (LPS), optionally wherein

the lysophospholipids are selected from lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), and lysophosphatidic acid (LPA), further optionally wherein

the phospholipids comprise one or more of the following:

i) from about 0 to about 5% LPC;

ii) from about 5 to about 20% LPE;

iii) from about 1 to about 15% LPS;

iv) from about 0 to about 10% PS;

v) from about 0 to about 5% PI; and

vi) from about 0 to about 5% PA.

3. The lipid according to claim 1 or 2, wherein the phospholipid esterified fatty acids comprise from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 2.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; from about 5.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; or from about 6 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% esterified C4 fatty acids; and/or

the phospholipid esterified fatty acids comprise from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 2.5 mol% to

about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 3.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 4 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; from about 4.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; or from about 5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 mol% esterified C6 fatty acids; and/or

from about 1.5% to 20% of the phospholipids are PC comprising esterified C4 fatty acids (C4-PC), and/or from about 1.5% to 20% of the phospholipids are PE comprising esterified C4 fatty acids (C4-PE), optionally wherein:

from about 1.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2.5% to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3.5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; or from about 5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% of the phospholipids are C4-PC; and/or

from about 1.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 2.5% to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 3.5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; from about 4.5 to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20%; or from about 5 to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% of the phospholipids are C4-PE.

4. The lipid according to any one of claims 1 to 3, wherein the phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C8 fatty acids; and/or

the phospholipid esterified fatty acids comprise C16 fatty acids; and/or C18 fatty acids.

5. The lipid according to any one of claims 1 to 4, wherein the phospholipid esterified fatty acids comprise:

from about 3 mol% to about 60 mol%; from about 5 mol% to about 55 mol%; from about 10 mol% to about 50 mol%; from about 10 mol% to about 40 mol%; from about 10 mol% to about 30 mol%; from about 10 mol% to about 60 mol%; from about 15 mol% to about 55 mol%; from about 15 mol% to about 50 mol%; from about 15 mol% to about 40 mol%; from about 15 mol% to about 30 mol%; from about 15 mol% to about 25 mol%; from about 20 mol% to about 60 mol%; from about 20 mol% to 55 mol%; from about 20 mol% to 45 mol%; from about 3 mol% to about 30 mol%; from about 5 mol% to about 25 mol%; or from about 10 mol% to about 20 mol% esterified C16 fatty acids;

and/or from 25 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol%; from about 30 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol%; from about 35 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol%; or from about 40 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90 mol% C18 esterified fatty acids, wherein

the C16 fatty acids optionally comprise C16:0 fatty acids, and/or the C18 fatty acids optionally comprise one or more or all of C18:0, C18:1, C18:2 and C18:3 fatty acids; and/or

the phospholipid esterified fatty acids comprise any one or more of i)-v):

i) from about 5 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 6 mol% to about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 7 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; from about 8 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; from about 9 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids; or from about 10 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C16:0 esterified fatty acids;

ii) from about 1 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 1.5 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2.5 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; or from about 3 mol% to about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol% C18:0 esterified fatty acids;

iii) from about 2 mol% to about 20, 25, 30, 35, 40, 45, 50, 55, 60 or 65 mol%; from about 5 mol% to about 20, 25, 30, 35, 40, 45, 50, 55, 60 or 65 mol%; from about 2 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 2 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 4 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 5 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; from about 6 mol% to about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol%; or from about 7 mol% to about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 30 mol% C18:1 esterified fatty acids;

iv) from about 20 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%;from about 25 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; from about 30 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; from about 35 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol%; or from about 40 mol% to about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 mol% C18:2 esterified fatty acids; and/or

v) from about 1 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 1.5 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; from about 2.5 mol% to

about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol%; or from about 3 mol% to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol% C18:3 esterified fatty acids, for example wherein the phospholipid esterified fatty acids comprise from about 5 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 25 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 1 mol% to about 10 mol% C18:3 fatty acids.

6. The lipid according to any one of claims 1 to 5, comprising monoacylglycerides, wherein the monoacylglycerides comprise monoacylglyceride esterified fatty acids, and wherein the monoacylglyceride esterified fatty acids comprise C4 fatty acids, C6 fatty acids or a combination thereof; optionally wherein the monoacylglyceride esterified fatty acids comprise at least 1.5, 2, 3, 4 or 5 mol% C4 fatty acids, C6 fatty acids or a combination thereof.

7. The lipid according to any one of claims 1 to 6, wherein

the lipid comprises at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% phospholipids (by weight); and/or

the lipid comprises no more than 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% monoacylglycerides; and/or

the lipid comprises no more than 15%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% triacylglycerides.

8. A composition comprising:

the lipid according to any one of claims 1 to 7; or

a lipid comprising phospholipids, wherein the phospholipids comprise phospholipid esterified fatty acids, wherein the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids, and wherein:

i) the phospholipids comprise from about 25% to 55% phosphatidylcholine (PC) and from about 15% to 35% phosphatidylethanolamine (PE);
ii) the phospholipid esterified fatty acids comprise from about 2 or 4 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 25 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 1 mol% to about 10 mol% C18:3 fatty acids;
iii) the composition comprises from about 20% to about 70% phospholipid; and/or
iv) the composition comprises one or more of carbohydrates, protein and ash, optionally wherein the carbohydrates and/or protein are soy, sunflower, cottonseed or rapeseed carbohydrates and/or protein.

9. The composition according to claim 8, wherein:

i) the total fat (by dry weight) of the composition is from about 30% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%;

from about 35% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 40% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 42% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 44% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 45% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 46% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; from about 48% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; or from about 50% to about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 70%, 71%, 72%, 73%, 74% or 75%; and/or

ii) at least or about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% of the total fat is phospholipids; and/or

iii) the total fatty acids comprises about 1.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 % C4 fatty acids; from about 2 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 25, 30 or 35 % C4 fatty acids; from about 2.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 25, 30 or 35 % C4 fatty acids; from about 3 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 3.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 4 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; from about 4.5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; or from about 5 % to about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or 35 mol% C4 fatty acids; and/or

iv) the total fatty acids comprises about 70% to about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 75% to about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 80% to about 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97 or 98% long chain fatty acids; from about 85% to about 90, 92, 93, 94 or 95 % long chain fatty acids; or from about 90% to about 93, 94, 95, 96, 97 or 98% long chain fatty acids.

10. The composition according to claim 8 or 9, wherein:

i) the composition comprises protein, optionally from about 1 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 2 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 3 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); from about 4 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); or from about 5 % to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20% protein (by weight); and/or

ii) the composition comprises carbohydrate, optionally from about 5% to about 20, 25, 30, 35, 40 or 50 % carbohydrate (by weight); from about 6 % to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 7% to about 20, 25, 30, 35, 40% carbohydrate (by weight); from about 8% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 9% to

about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); from about 10% to about 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); or from about 5% to about 10, 20, 25, 30, 35, 40, or 50% carbohydrate (by weight); and/or

iii) the composition comprises ash.

11. The composition according to any one of claims 8 to 10, comprising:

i) an aqueous component and a hydrophobic component, optionally wherein the hydrophobic component comprises a non-polar lipid; optionally wherein the non-polar lipid comprises triacylglycerides, optionally wherein the non-polar lipid is in the form of a vegetable oil; and/or

ii) lipid droplets;

optionally wherein the phospholipids are present in the composition in an amount of at least 0.005% or 0.01% by weight.

12. The composition according to any one of claims 8 to 11, further comprising one or more bioactive agents, optionally wherein said bioactive agents are selected from steroids, anti-oxidants, vitamins, minerals, fatty acids, amino acids, prebiotics, peptides, proteins, enzymes, drugs, antibodies, and hormones.

13. A delivery vehicle for delivering one or more components to a subject, for example for delivery of one or more bioactive agents, the delivery vehicle comprising the composition of claim 11 or 12 wherein the composition comprises lipid droplets, optionally wherein said bioactive agents are selected from steroids, anti-oxidants, vitamins, minerals, fatty acids, amino acids, prebiotics, peptides, proteins, enzymes, drugs, antibodies, and hormones.

14. A food, beverage or feedstuff comprising:

a) a lipid comprising phospholipids, said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, optionally wherein:

the phospholipid esterified fatty acids comprise from about 1.5% to about 35% mol% C4 fatty acids or from about 4% to about 35% mol% C4 fatty acids; and/or

the phospholipid esterified fatty acids comprise from about 1.5 or 4 mol% to about 35 mol% C4 fatty acids, from about 10 mol% to about 30 mol% C16:0 fatty acids, from about 1 mol% to about 10 mol% C18:0 fatty acids, from about 3 mol% to about 20 mol% C18:1 fatty acids, from about 30 mol% to about 70 mol% C18:2 fatty acids, and from about 11 mol% to about 15 mol% C18:3 fatty acids; or

b) the lipid according to any one of claims 1 to 7; or

c) the composition according to any one of claims 8 to 12.

15. The food, beverage or feedstuff of claim 14, wherein the composition of any one of claims 8 to 12 is present in the food, beverage or feedstuff in an amount of up to 5%, 4.5%, 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, 1%, 0.5%, 0.4%, 0.3%, 0.2% or 0.1% by weight and/or the composition of any one of claims 8 to 12 is present in the food, beverage or feedstuff in an amount of at least 0.01%,

0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.55%, 0.6%, 0.65%, 0.7%, 0.75%, 0.8%, 0.85%, 0.9%, 0.95% or 1% by weight; or

the lipid according to any one of claims 1 to 7 is present in the food, beverage or feedstuff in an amount of up to 2.5%, 2%, 1.5%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% or 0.01% by weight and/or

the lipid according to any one of claims 1 to 7 is present in the food, beverage or feedstuff in an amount of at least 0.001%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45% or 0.5% by weight.

16. A method of producing a food, beverage or feedstuff, the method comprising mixing the lipid according to any one of claims 1 to 7 or the composition of any one of claims 8 to 12 with at least one other consumable ingredient.

17. Use of the lipid of any one of claims 1 to 7 or the composition of any one of claims 8 to 12 to produce a food, beverage or feedstuff.

18. The food, beverage or feedstuff of claim 15 or the method of claim 16 or the use of claim 17, wherein:

the food, beverage or feedstuff contains no components derived from an animal and/or the food, beverage or feedstuff is a dairy product substitute, optionally wherein the food, beverage or feedstuff is a dairy-free milk, dairy-free yoghurt, dairy-free cheese, dairy-free cream, dairy-free butter, dairy-free margarine, dairy-free chocolate or dairy-free ice cream; or

the food, beverage or feedstuff is a dairy product, optionally wherein the food, beverage or feedstuff is a milk, yoghurt, butter, cheese, cream, ice cream or chocolate, for example a reduced fat milk.

19. A process for preparing a lipid comprising phospholipids, said phospholipids comprising phospholipid esterified fatty acids, wherein said phospholipid esterified fatty acids comprise at least 1.5, 2, 2.5 or 3 mol% C4 fatty acids, C6 fatty acids or a combination thereof, wherein:

the process comprises contacting a source of phospholipids with: a) a triacylglyceride or a diacylglyceride comprising esterified C4 and/or C6 fatty acids, butyric acid or caproic acid, such as tributyrin; and b) an optionally immobilized enzyme suitable for effecting transesterification of the phospholipid with C4 and/or C6 fatty acids, for example a lipase, a phospholipase and/or a triacylglyceride lipase, for a time and under conditions sufficient to effect such transesterification, to thereby produce the lipid; and

the source of phospholipids is a lecithin;

optionally wherein the lipid is as defined in any one of claims 1 to 7; and optionally wherein

the lecithin is a plant lecithin, such as a soy lecithin, sunflower lecithin, cottonseed lecithin or rapeseed lecithin, egg yolk lecithin, yeast lecithin or fungus lecithin; and optionally wherein

the process further comprises purifying the lipid, optionally by acetone and/or ethanol precipitation or extraction; and optionally wherein

the composition of any one of claims 8 to 12 is produced.

20. A lipid prepared by the process according to claim 19.