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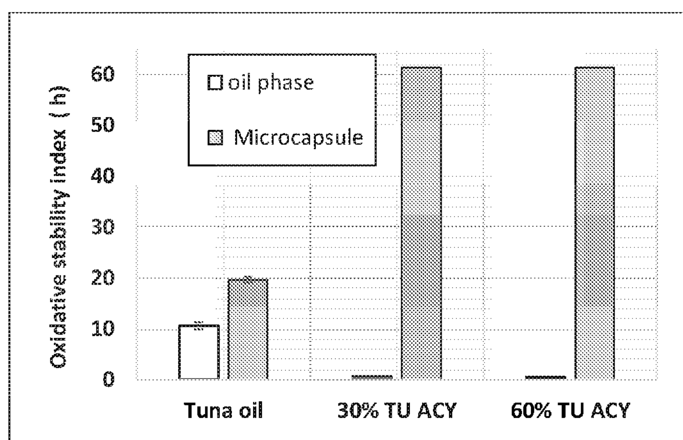
(54) Title: MICROENCAPSULATED OMEGA-3 POLYUNSATURATED FATTY ACID GLYCERIDE COMPOSITIONS AND PROCESSES FOR PREPARING THE SAME

Figure 9



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(57) Abstract: The invention provides a microencapsulated omega-3 polyunsaturated fatty acids glyceride composition, products comprising such compositions, uses of such compositions, and processes for preparing such compositions, wherein the glycerides comprise at least one of monoglycerides and diglycerides, and are microencapsulated by preparing a mixture comprising an emulsion wherein droplets of the glycerides are dispersed in an aqueous continuous phase, the mixture further comprising one or more polymer components, and microencapsulating the droplets of the glycerides with the one or more polymer components by a method selected from spray-drying the mixture and inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets.

[Continued on next page]

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Microencapsulated omega-3 polyunsaturated fatty acid glyceride compositions and processes for preparing the same

Technical Field

[1] The present invention relates to microencapsulated glyceride compositions enriched in omega-3 polyunsaturated fatty acids, processes for preparing such compositions, products comprising such compositions and uses of such compositions. Specifically, the encapsulated glycerides comprise at least one of monoglycerides and diglycerides.

Background of Invention

[2] Polyunsaturated fatty acids (PUFA) such as the omega-3 fatty acids are vital to everyday life and function. For example, the beneficial effects of omega-3 fatty acids like *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) on lowering serum triglycerides are now well established. These compounds are also known for other cardioprotective benefits. (*See* e.g., Dyrberg, *et al.*, in: ω-3 Fatty Acids: Prevention and Treatment of Vascular Disease, Kristensen, *et al.*, eds., Bi & Gi Publ., Verona-Springer-Verlag, London, pp. 217-26, 1995; O'Keefe and Harris, *Am. J. Cardiology* 2000, 85: 1239-41; Radack et al., "The effects of low doses of omega-3 fatty acid supplementation on blood pressure in hypertensive subjects: a randomized controlled trial." *Arch. Intern. Med.* 1991, 151:1173-1180). Indeed, the American Heart Association has also reported that omega-3 fatty acids can reduce cardiovascular and heart disease risk. Other benefits of PUFAs are those related to the prevention and/or treatment of inflammation, neurogenerative diseases, and cognitive development. (*See* e.g., Sugano, Michihiro, "Balanced intake of polyunsaturated fatty acids for health benefits." *J. Oleo Sci.* 2001, 50(5):305-311). Diets rich in PUFA's like omega-3 fatty acids have also been shown to have beneficial effects for heart disease, cancer, arthritis, allergies, and other chronic diseases. (*See* e.g., The American Heart Association, Scientific Statement, "Fish Consumption, Fish Oil, Omega-3 Fatty Acids and Cardiovascular Disease," November 2002; Appel et al., "Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical

trials." *Arch. Intern. Med.* 1993, 153(12):1429-1438; GISSI-Prevenzione Investigators, "Dietary supplementation with omega-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial." *Lancet* 1999, 354:447-455.)

5 [3] Because of the strong clinical support for the role of EPA and DHA in maintaining good health and preventing disease throughout the human life cycle, several international groups, including the American Heart Association, the Australian National Health and Medical Research Council, the Japan Ministry of Health and Welfare and the British Nutrition Foundation, have made recommendations for 10 omega-3 intake, and for EPA and DHA specifically. These range from consuming two fatty fish meals per week for a healthy population (equivalent to approximately 500 mg/day of omega-3 PUFA's), to consuming 2 to 4 g of EPA and DHA daily for the medical management of elevated blood triacylglycerols or hypertriglyceridemia.

[4] In many modern societies, however, a substantial proportion of the 15 population consumes a diet containing a large amount of saturated fatty acids and a low proportion of PUFA. Despite international recommendations for EPA and DHA intake, the estimated consumption in North America and European countries is well below current recommendations, with estimated intakes in the range of 20 to 300 mg/day depending upon age group and country.

20 [5] The nutritional gap between recommended and actual consumption of omega-3 fatty acids, coupled with consumer desire to improve overall health and nutrition, has created a large global market for omega-3 containing functional foods and nutritional supplements. Various foodstuffs fortified with omega-3 fatty acids, including bread, milk, yoghurt and orange juice, are currently commercially available. 25 Furthermore, fortification of infant formula with omega-3 fatty acids, notably DHA which is an important component of breast milk, is standard industry practice.

[6] However, PUFA's cannot be added successfully to the majority of food products unless they have been stabilized against oxidative degradation. EPA and DHA are inherently unstable, reacting rapidly with oxygen in a free radical reaction 30 process (autoxidation) in which hydroperoxides (ROOH) are formed through reaction at an allylic center (CH_2 adjacent to a double bond). The hydroperoxides that result

readily break down to a range of volatile aldehydes. These cause the undesirable odour and taste associated with rancid oils. Significant exposure of omega-3 oils to oxygen lead to a gradual decrease in EPA and DHA levels. Moreover, even trace amounts of the aldehyde oxidation products can be detected by human taste and smell. Therefore, any method aimed at stabilizing fish or algal oils for use in fortified food products must be very effective and avoid the formation of even low ppm levels of oxidation products. Various types of antioxidants have been used for the chemical stabilisation of EPA and DHA, but these are not adequate to enable sensory stabilisation of these PUFA's in many food and beverage applications.

[7] Microencapsulation is a technique for converting liquids into solid form, often as free-flowing powders, by encapsulating tiny droplets of the liquid within a solid shell or matrix. It has been used in the food and pharmaceuticals industries to mask unpleasant flavours and odours of microencapsulated ingredients, to protect ingredients from oxidation and other unwanted reactions, thereby extending shelf life, and to control the release of substances such as enzymes, flavours, nutrients and drugs. Microencapsulation is thus an important approach for improving the functionality of food additives and expanding the range of food ingredients useful for various applications.

[8] Spray drying is a widely used microencapsulation technique in the food industry. In this process an oil-in-water emulsion of the target ingredient is spray-dried in the presence of protein or carbohydrate so that the oil is embedded within a solid matrix. The limitations of this technology are that the particle size is normally large, the oil payload is low (normally less than 30%), and the level of surface oil is high. A low oil payload implies that a larger mass of microcapsules must be added to the fortified product to achieve the required dose, increasing costs and potentially adversely affecting one or more properties of the product such as mouthfeel. Surface oil, being on or close to the outer surface of the microcapsule, is essentially unprotected against oxidation, leading to rapid sensory deterioration.

[9] Another approach previously used to stabilise PUFA's is to microencapsulate the PUFA-containing oil in single- or multi-core microcapsules with walls formed from a coacervate. Coacervation is a process whereby a

macromolecular solution is separated into two immiscible phases: a macromolecule-rich coacervate phase and a macromolecule-lean phase. In a simple coacervation process, a solution of a single macromolecule phase separates, for example after the macromolecule has been chemically modified to promote polymer-polymer interactions over polymer-water interactions, or by adding another miscible non-solvent or a salt, or by changing the temperature. In a complex coacervation process, the coacervate is formed when two different macromolecules coalesce when conditions are adjusted to induce electrostatic interactions between the oppositely charged macromolecules.

[10] In both cases, the microcapsules are formed by preparing an emulsion of the PUFA-containing oil in an aqueous solution of the macromolecules, and then inducing coacervation so that the coacervate phase coalesces at the oil-water interface of the emulsion to form a layer around the oil droplets. The coacervate may then be solidified to form shells encapsulating the oil droplets by various techniques, including spray-drying the emulsion or adding a cross-linking agent.

[11] Complex coacervation has previously been used to microencapsulate PUFA-containing oils. In a preferred approach, the technique may be used to form multi-core microcapsules with high oil payloads and low surface oil content. (*See* U.S. 6,974,592). Primary microcapsules of coacervate-coated oil droplets are induced to agglomerate, and the conditions are subsequently adjusted, for example by lowering the temperature, so that a further layer of coacervate is deposited to form an outer shell surrounding the agglomerate. A number of bio-polymer combinations have been used to form complex coacervates for microcapsule shells, including gelatin / polyphosphate and whey protein / gum arabic, with coacervation typically being induced by adjusting the pH of the aqueous solution of the biopolymers.

[12] Omega-3 fatty acids are often derived from marine oils, microbial, and/or algal oils. Such sources provide the PUFA in a triglyceride form where other undesired, fatty acids (e.g. saturated fatty acids) are present alongside a desired PUFA in the triglyceride molecule. Most native fish oils contain no more than 30% of EPA and DHA. For example, the primary sources of most commercially used omega-3 fish oil is anchovy (Engraulis ringens) and sardine (Sardinops sagax sagax) oils that

contain 15–22% of EPA and 9–15% of DHA. However, it is preferred that omega-3 fatty acid oils supplements for fortification should contain significantly higher levels of EPA and DHA. This minimises the amount of supplement required in the product for a given dosage, which may be functionally important. Furthermore, microencapsulation can be a significant, or even predominant, contributor to the total cost of a suitably stabilised PUFA-containing supplement. Thus, microcapsules prepared with EPA- and DHA enriched oils may be more cost-effective than those prepared from native fish-oil, even when the native oil is a lower-cost source of EPA and DHA than the PUFA oil concentrate.

[13] Several methods of producing PUFA concentrates from oils, such as marine, microbial, and/or algal oils, have been disclosed. Commonly, chemical ethanolysis is used to completely liberate the PUFA's from the triglycerides as ethyl esters. The ethyl esters may then be fractionated by distillation or urea complexation. However, ethyl ester PUFA concentrates are reported to be less bio-available than the triglyceride form, and regulations in some jurisdictions require PUFA supplements or additives to be provided as glycerides, replicating the natural form in foods. The fractionated PUFA ethyl esters are thus typically converted into the triglyceride form by trans-esterification with glycerol, using chemical or enzymatic methods. In this way, triglycerides with extremely high levels of EPA and DHA can be produced.

[14] Another reported approach for obtaining PUFA concentrates from native fish oils is via a two-step selective hydrolysis / re-esterification process. (*See* U.S. 8,420,439). In the first step, short-chain and/or saturated fatty acids in the triglyceride native oil are selectively hydrolised with a lipase, thereby increasing the EPA and DHA content in the partially hydrolysed acylglycerol residue. The free fatty acids liberated in the hydrolysis are then removed. In the second step, the free hydroxyl groups in the acylglycerol residue are re-esterified with EPA and/or DHA in a suitable form (either as the free fatty acid or the ethyl ester) to give triglycerides containing further increased levels of EPA and DHA. This approach has the benefit of preserving the regioselectivity of the original EPA and DHA residues in the starting triglycerides, thus more closely resembling a natural fish oil than a fully synthetic analogue prepared from glycerol.

[15] In both of these approaches, the PUFA concentrate is provided as triglycerides. This most closely resembles the natural form of PUFA's, but more importantly also enables the maximum EPA and DHA content to be achieved per unit mass of the final concentrate.

5 [16] PUFA oil concentrates are known to be less oxidatively stable than the native fish oils from which they are produced. This may be due both to the higher EPA and DHA content itself (these fatty acids being particularly susceptible to oxidation due to the presence of multiple allylic centres), and the greater fraction of the EPA and DHA being present on the more exposed sn-1,3-positions compared to 10 native oils, in which DHA in particular is more prevalent on the more protected sn-2-position of the glycerides (see e.g. Akanbi et al, Food Chemistry, 2013, 138, 615).

[17] There is therefore a need to develop new approaches to stabilising omega-3 PUFA-containing oils, and in particular omega-3 polyunsaturated fatty acid glyceride concentrates, against oxidation. Such approaches may offer a variety of potential 15 benefits, including reduced amounts of supplement required to achieve a target dosage of PUFA, improved shelf-life of products fortified with PUFA's, and development of new PUFA-fortified foods and beverages previously inaccessible due to inadequate stability of PUFA concentrates.

[18] A reference herein to a patent document or other matter which is given as 20 prior art is not to be taken as an admission that the document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Summary of Invention

[19] We have discovered a process for microencapsulating glycerides enriched 25 in omega-3 polyunsaturated fatty acids such that enhanced stability against oxidative degradation can be achieved. In particular, we have found that oxidative stability may be advantageously and unexpectedly enhanced when glycerides comprising a fraction of monoglycerides and / or diglycerides are microencapsulated. Microencapsulation is performed by preparing an oil-in-water emulsion of droplets of 30 the glycerides in an aqueous phase, together with one or more polymer components,

and subsequently encapsulating the glycerides with the one or more polymer components by spray drying or coacervation techniques.

[20] In some embodiments, the microencapsulated glycerides comprise at least one of monoglycerides and diglycerides in a total amount of at least 5%, based on the total mass of the glycerides. In some embodiments, the microencapsulated glycerides are formed by partially hydrolysing triglycerides with a lipase, and removing free fatty acids liberated from the triglycerides.

[21] Surprisingly, we have found that the microencapsulated glycerides prepared according to the process of the invention are more stable against oxidation than microencapsulated triglycerides, where the triglycerides have similar composition to the glycerides except for the fraction of monoglycerides and / or diglycerides. This may be so even when the concentration of inherently oxidation-sensitive omega-3 PUFA is higher in the glycerides than in the corresponding triglycerides, for example when the glycerides is an omega-3 concentrate formed by selectively removing fatty acids other than omega-3 PUFA's from triglycerides by lipase hydrolysis. Furthermore, the surprisingly enhanced stability of the microencapsulated glycerides comprising a fraction of monoglycerides and / or diglycerides, relative to the corresponding microencapsulated triglycerides, may be obtained by following the process of the invention even when the non-encapsulated glycerides are substantially more sensitive to oxidation than the non-encapsulated triglycerides.

[22] In accordance with one aspect the invention provides a process for preparing a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, the process comprising:

providing glycerides comprising at least one of monoglycerides and diglycerides in a total amount of at least 5%, preferably at least 10%, more preferably at least 20%, most preferably at least 30% based on the total mass of the glycerides, wherein the glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides,

preparing a mixture comprising an emulsion wherein droplets of the glycerides are dispersed in an aqueous continuous phase, the mixture further comprising one or more polymer components; and

microencapsulating the droplets of glycerides with the one or more polymer components by a method selected from spray-drying the mixture and inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets.

5 [23] In accordance with another aspect the invention provides a process for preparing a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, the process comprising:

contacting triglycerides with a lipase under aqueous conditions for a time and at a temperature sufficient to partially hydrolyse the triglycerides to form partially 10 hydrolysed triglycerides;

removing free fatty acids from the partially hydrolysed triglycerides to provide glycerides comprising at least one of monoglycerides and diglycerides, wherein the glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides;

15 preparing a mixture comprising an emulsion wherein droplets of the glycerides are dispersed in an aqueous continuous phase, the mixture further comprising one or more polymer components; and

microencapsulating the droplets of the glycerides with the one or more polymer components by a method selected from spray-drying the mixture and 20 inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets.

[24] In some embodiments, the lipase is *Thermomyces lanuginosus* lipase. In some embodiments, the triglycerides are selected from the group consisting of fish oils, microbial oils and algal oils, and may be fish oil selected from tuna oil and 25 anchovy oil.

[25] In some embodiments, the one or more polymer components comprise a protein polymer component and a polyanionic polymer component, and the glycerides are microencapsulated by inducing complex coacervation of the protein polymer component and the polyanionic polymer component to form primary shells of complex 30 coacervate encapsulating droplets of the glycerides.

[26] In some embodiments, the process further comprises producing agglomerations of the primary shells; and cooling the mixture to form secondary shells of complex coacervate encapsulating the agglomerations.

[27] In accordance with another aspect the invention provides a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, prepared according to any of the embodiments disclosed herein.

[28] In accordance with another aspect the invention provides a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, the composition comprising:

glycerides comprising at least one of monoglycerides and diglycerides in a total amount of at least 5%, preferably at least 10%, more preferably at least 20%, most preferably at least 30% based on the total mass of the glycerides, wherein the glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides;

an encapsulant which encapsulates droplets of the glycerides, the encapsulant comprising one or more polymer components.

[29] In some embodiments, the encapsulant is a complex coacervate formed as primary shells encapsulating droplets of the glycerides, the complex coacervate comprising a protein polymer component and a polyanionic polymer component. In some embodiments, agglomerations of the primary shells are encapsulated with secondary shells of the complex coacervate.

[30] In accordance with another aspect the invention provides a nutritional supplement, functional food, medical food or infant formula comprising the microencapsulated omega-3 polyunsaturated fatty acid glyceride composition according to any of the embodiments disclosed herein.

[31] In accordance with another aspect the invention provides use of a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition according to any of the embodiments disclosed herein, for the treatment or prevention of a disease or medical condition, wherein the disease or medical condition is treatable or preventable by ingestion of omega-3 polyunsaturated fatty acids.

[32] Where the terms “comprise”, “comprises” and “comprising” are used in the specification (including the claims) they are to be interpreted as specifying the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

5 [33] Further aspects of the invention appear below in the detailed description of the invention.

Brief Description of Drawings

[34] Embodiments of the invention will herein be illustrated by way of example only with reference to the accompanying drawings in which:

10 [35] Figure 1 is a graph showing the effect of pH on hydrolysis degree of anchovy oil over time periods of 12, 15.5 and 19 hours.

[36] Figure 2 is a graph showing the effect of hydrolysis reaction time on the hydrolysis degree of anchovy oil, at a pH of 7.5.

[37] Figure 3 is a graph showing the major fatty acid composition of native 15 anchovy oil and partially hydrolysed anchovy oils with 30% and 60% hydrolysis degree.

[38] Figure 4 shows a series of images of complex coacervate covered primary shells and final product microcapsules, obtained when microencapsulating native anchovy oil and partially hydrolysed anchovy oils with 30% and 60% hydrolysis 20 degree.

[39] Figure 5 shows SEM images of final product microcapsules obtained when microencapsulating native anchovy oil and partially hydrolysed anchovy oils with 30% and 60% hydrolysis degree.

[40] Figure 6 shows graphs of the Rancimat accelerated oxidation test results 25 for native anchovy oil and partially hydrolysed anchovy oils with 30% and 60% hydrolysis degree, both as free oil and microencapsulated compositions, wherein the arrows indicate inflection points corresponding to the Oxidative Stability Index values.

[41] Figure 7 is a graph showing the effect of hydrolysis reaction time on the hydrolysis degree of tuna oil, at a pH of 7.5.

[42] Figure 8 is a graph showing the major fatty acid composition of native tuna oil and partially hydrolysed tuna oils with 30% and 60% hydrolysis degree.

5 [43] Figure 9 is a graph showing the Oxidative Stability Index values, as determined by Rancimat accelerated oxidation tests, for native tuna oil and partially hydrolysed tuna oils with 30% and 60% hydrolysis degree, both as free oil and microencapsulated compositions.

[44] Figure 10 is a graph showing the Oxidative Stability Index values, as 10 determined by Rancimat accelerated oxidation tests, for microencapsulated glyceride compositions, wherein the microencapsulated glycerides comprise a series of mixtures of native tuna oil and partially hydrolysed tuna oil with 30% hydrolysis degree.

Detailed Description

15 [45] The present invention relates to a process for microencapsulating glycerides enriched in omega-3 polyunsaturated fatty acids, specifically comprising at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides. Importantly, the microencapsulated glycerides comprise at least one of monoglycerides and diglycerides. The glycerides may comprise at least one of 20 monoglycerides and diglycerides in a total amount of at least 5% based on the total mass of the glycerides.

[46] The glycerides may be provided by contacting triglycerides with a lipase such as *Thermomyces lanuginosus* lipase under aqueous conditions for a time and at a temperature sufficient to partially hydrolyse the triglycerides to form partially 25 hydrolysed triglycerides, and removing free fatty acids from the partially hydrolysed triglycerides.

[47] A mixture comprising an emulsion of droplets of the glycerides dispersed in an aqueous continuous phase is then prepared, the mixture further comprising one or more polymer components.

[48] The droplets of the glycerides are then microencapsulated by a method selected from spray-drying the mixture and inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets.

[49] The one or more polymer components may comprise a protein polymer component and a polyanionic polymer component, and the droplets of glycerides are microencapsulated by inducing complex coacervation of the protein polymer component and the polyanionic polymer component to form primary shells of complex coacervate encapsulating the droplets. Moreover, agglomerations of the primary shells may be produced, and secondary shells of complex coacervate encapsulating the agglomerations may be formed by cooling the mixture.

Glycerides

[50] The present invention relates to methods for preparing a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, microencapsulated omega-3 polyunsaturated fatty acid glyceride compositions, products comprising such compositions, and uses of such compositions.

[51] As used herein, a fatty acid means an aliphatic monocarboxylic acid. The aliphatic group may be saturated or unsaturated, and typically comprises at least 4 carbon atoms. The carboxyl group of a fatty acid may be in either protonated or deprotonated form (i.e. RCO_2H or RCOO, where R is the aliphatic group). This convention and nomenclature is typically used by those in the field.

[52] As used herein, an omega-3 polyunsaturated fatty acid is a fatty acid wherein the aliphatic group comprises two or more carbon–carbon double bonds and contains CH_3-CH_2-CH=CH- as its terminus. Examples of omega-3 polyunsaturated fatty acids include include, but are not limited to, linolenic acid (18:3ω3), octadecatetraenoic acid (18:4ω3), eicosapentaenoic acid (20:5ω3) (EPA),

docosahexaenoic acid (22:6ω3) (DHA), docosapentaenoic acid (22:5ω3) (DPA), derivatives thereof and mixtures thereof.

[53] As used herein, glycerides are fatty acid esters of glycerol (propane-1,2,3-triol). Glycerides consist of triglycerides, diglycerides (including both 1,2- and 1,3-diglycerides) and monoglycerides (including both 1- and 2-monoglycerides). Glycerides are also known as acylglycerols.

[54] As used herein, omega-3 polyunsaturated fatty acid glycerides are glycerides comprising omega-3 polyunsaturated fatty acids, but which may also comprise other fatty acids.

[55] The microencapsulated glycerides comprise at least one of monoglycerides and diglycerides. It will be appreciated by a person skilled in the art that any residual glycerides consist of triglycerides.

[56] In some embodiments, the microencapsulated glycerides comprise both monoglycerides and diglycerides. In some embodiments, the microencapsulated glycerides comprise monoglycerides but no diglycerides, or diglycerides but no monoglycerides.

[57] The microencapsulated glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides. In some embodiments, the microencapsulated glycerides comprise at least 40%, or 50%, or 60% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides. It will be appreciated by a person skilled in the art that the residual fatty acids in the glycerides may be any other fatty acids, including saturated acids, mono-unsaturated fatty acids, and other polyunsaturated fatty acids.

Glycerides comprising at least 5% monoglycerides and/or diglycerides

[58] In some embodiments, the glycerides comprise at least one of monoglycerides and diglycerides in a total amount of at least 5%, or 10%, or 20%, or 30% based on the total mass of the glycerides.

[59] In some embodiments, the microencapsulated glycerides comprise both of monoglycerides and diglycerides in a total amount of at least 5%, or 10%, or 20%, or

30% based on the total mass of the glycerides. In some embodiments, the microencapsulated glycerides comprise monoglycerides but no diglycerides, or diglycerides but no monoglycerides in a total amount of at least 5%, or 10%, or 20%, or 30% based on the total mass of the glycerides.

5 [60] Glycerides comprising at least one of monoglycerides and diglycerides in a total amount of at least 5% based on the total mass of the glycerides and at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides may be prepared by any suitable means. In some embodiments, the glycerides may be formed by esterification of glycerol with free fatty acids comprising omega-3 10 polyunsaturated fatty acids. Alternatively, the glycerides may be formed by transesterification of glycerol with fatty acid esters comprising omega-3 polyunsaturated fatty acid esters, for example the ethyl esters of fatty acids. In these embodiments, the glycerol may intentionally be incompletely esterified so as to produce the required fraction of monoglycerides and/or diglycerides in the 15 esterification reaction product. This may be done, for example, by adding sub-stoichiometric amounts of the free fatty acids or fatty acid esters to the glycerol, or by selecting the reaction conditions to prevent complete reaction.

[61] In another embodiment, the glycerides comprising at least one of monoglycerides and diglycerides in a total amount of at least 5% based on the total 20 mass of the glycerides and at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides may be prepared by a process comprising transesterification of glycerides with fatty acids comprising omega-3 polyunsaturated fatty acids.

[62] In some embodiments, the glycerides comprising at least one of 25 monoglycerides and diglycerides in a total amount of at least 5% based on the total mass of the glycerides and at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides are prepared by hydrolysing triglycerides. Hydrolysis may be performed by any suitable means, including chemical and enzymatic methods. Examples of chemical hydrolysis methods include 30 saponification, i.e. reacting the triglycerides with a base such as sodium hydroxide. Acids may also be used to catalyse the hydrolysis of triglycerides. Examples of

enzymatic hydrolysis methods include contacting the triglycerides with an esterase. Suitable esterases include, but are not limited to, lipases.

Glycerides prepared by contacting triglycerides with a lipase, and removing free fatty acid

5 [63] In some embodiments, the glycerides are prepared by contacting triglycerides with a lipase under aqueous conditions for a time and at a temperature sufficient to partially hydrolyse the triglycerides to form partially hydrolysed triglycerides, and removing free fatty acids from the partially hydrolysed triglycerides.

Triglycerides

10 [64] The triglycerides may be any suitable triglycerides comprising omega-3 polyunsaturated fatty acids. In one embodiment, the triglycerides are a natural product, such as that derived from fish oil, microbial oil, algal oil, or a combination thereof. In another embodiment, the triglycerides are a synthetic product, such as a product derived from the esterification of a glycerol with fatty acids comprising omega-
15 3 polyunsaturated fatty acids. In one such embodiment, the triglycerides are synthetic fatty acid triglycerides, wherein the fatty acids consist of omega-3 polyunsaturated fatty acids. In another embodiment, the starting oil composition is a combination of a natural product and a synthetic product.

[65] In one embodiment, the triglycerides are derived from marine oils, such as
20 fish oil. Such oils typically contain mixtures of saturated and unsaturated fatty acids, esters, and glycerides thereof. Any fish oil comprising omega-3 polyunsaturated fatty acid-containing triglycerides may be used. Examples of suitable fish oils include, but are not limited to, Atlantic fish oil, Pacific fish oil, Mediterranean fish oil, light pressed fish oil, alkaline treated fish oil, heat treated fish oil, light and heavy brown fish oil,
25 bonito oil, pilchard oil, tuna oil, sea bass oil, halibut oil, spearfish oil, barracuda oil, cod oil, menhaden oil, sardine oil, anchovy oil, capelin oil, Atlantic cod oil, Atlantic herring oil, Atlantic mackerel oil, Atlantic menhaden oil, salmon oil, and shark oil, including mixtures and combinations thereof. Non-alkaline treated fish oil is also suitable. In some embodiments, the fish oils are tuna oil and anchovy oil. Other
30 marine oils suitable for use include, but are not limited to, squid oil, cuttle fish oil,

octopus oil, krill oil, seal oil, whale oil, and the like, including mixtures and combinations thereof.

[66] In another embodiment, the triglycerides are, or are derived from, non-marine oils, including microbial oil, algal oil (e.g. oil from a dinoflagellate such as Crypthecodinium cohnii), fungal oil (e.g. oil from Thraustochytrium, Schizochytrium, or a mixture thereof) and plant oil, including mixtures and combinations thereof.

[67] The triglycerides may comprise, in addition to omega-3 polyunsaturated fatty acids, a plurality of other acids including saturated and unsaturated fatty acids, and even short chain carboxylic acids. Furthermore, it is not excluded that monoglycerides and/or diglycerides may already be present together with the triglycerides in the oil composition that is contacted with the lipase.

Lipase-catalysed hydrolysis

[68] The triglycerides are contacted with a lipase under aqueous conditions for a time and at a temperature sufficient to partially hydrolyse the triglycerides to form partially hydrolysed triglycerides.

[69] Prior to contacting the triglycerides with the lipase, an oil composition comprising the triglycerides to be partially hydrolysed may optionally be washed with water and/or a pH buffer. One or more washes with the same or varying wash compositions and conditions may be performed. In one embodiment, the oil composition is washed with 60°C water. In another embodiment, the oil composition is twice washed with 60°C water. The choice of wash solution (e.g., water, pH buffer, or other wash liquid), the quantity thereof, the wash temperature and the duration of the wash may be selected depending upon the starting oil composition and the desired outcome, for example the desired purity of the washed oil composition. A pH buffer wash solution, if used, may comprise any suitable buffer, and the pH of the buffer wash solution may be from about 7 to about 12. In one embodiment, a pH 10 buffer solution comprising a potassium carbonate-potassium borate-potassium hydroxide buffer (0.05 M) is selected. After an optional washing step, the aqueous fraction of the wash may optionally be separated and removed from the oil composition.

[70] As will be appreciated by a person skilled in the art, a hydrolysis reaction of an ester refers to the overall reaction of the ester functionality with water, resulting in formation of a carboxylic acid and an alcohol. A hydrolysis reaction of a glyceride molecule therefore liberates a free fatty acid, leaving a vacant hydroxyl group on the glyceride backbone. As used herein, triglycerides are partially hydrolysed when hydrolysis has not proceeded to completion to form exclusively glycerol and free fatty acids. The reaction product when triglycerides are partially hydrolysed thus comprises at least one of monoglycerides and diglycerides. However, it is not implied that each individual triglyceride molecule in the triglycerides is partially hydrolysed: partially hydrolysed triglycerides may, and typically will, comprise unreacted triglyceride molecules in combination with the monoglycerides and/or diglycerides. Furthermore, partial hydrolysis of triglycerides does not imply that each individual triglyceride molecule is at most partially hydrolysed: partially hydrolysed triglycerides may comprise glycerol molecules (i.e. fully hydrolysed triglyceride) in combination with the monoglycerides and/or diglycerides. In some embodiments, partially hydrolysed triglycerides may comprise each of, monoglycerides, diglycerides and triglycerides.

[71] The triglycerides are partially hydrolysed by contacting the triglycerides with a lipase under aqueous conditions. The lipase may be in liquid form. However, immobilised lipases may also be utilised. Lipases have been reported to catalyse the hydrolysis of lipids at a water-lipid interface (see for example Olusegun et al, New Biology 2011, 28, 738-745; Akanbi et al, International Food Research Journal 2010, 17, 45-53). Therefore, the triglycerides in the form of an oil, or an oil composition comprising the triglycerides to be partially hydrolysed, will typically be contacted with an aqueous phase comprising the lipase. The aqueous phase may be an aqueous solution of a water-soluble lipase. An aqueous solution of lipase can be prepared by mixing a quantity of the lipase enzyme in liquid form with water. The concentration and amount of the aqueous lipase solution can vary, depending on the required degree of hydrolysis, the desired reaction time, and the susceptibility of the triglycerides to hydrolysis.

[72] In one embodiment, the liquid lipase is a food-grade lipase. In another example, the lipase is at least one of a kosher and/or halal-certified food grade lipase.

[73] In one embodiment, the lipase enzyme comprises *Thermomyces lanuginosus*, such as LIPOZYME™ TL100 (available from Novozymes A/S, Bagsvaerd, Denmark). This enzyme has the sequence MRSSLVLFFVSAWTALASPIRREVSQDLFNQFNLFAQYSAAAYCGKNNDAPAGTNIT
5 CTGNACPEVEKADATFLYSFEDSGVGDVTGFLALDNTNKLIVLSFRGSRSIENWIGN LNFDLKEINDICSGCRGHDGFTSSWRSVADTLRQKVEDAVREHPDYRVVFTGHSLG GALATVAGADLRGNGYDIDVFSYGAPRVGNRAFAEFLTVQTGGTLYRITHTNDIVPR LPPREFGYSHSSPEYWIKSGTLVPVTRNDIVKIEGIDATGGNNQPNIPDIPAHLWYFG LIGTCL (SEQ ID NO:1). Enzymes with a sequence homology of at least 80, at least
10 85, at least 90, at least 95, at least 97, and at least 99% homology to SEQ ID NO: 1 are also contemplated herein for use in the disclosed methods.

[74] The use of liquid *Thermomyces lanuginosus* enzyme allows for efficient hydrolysis and may thus reduce the amount of enzyme needed to partially hydrolyse the triglycerides. The amount of *Thermomyces lanuginosus* needed can be less than
15 50%, or 25% or 10% of the amount of other lipase enzymes need to achieve the partial hydrolysis. Typically, the amount of *Thermomyces lanuginosus* used can be from about 0.01% to about 3.0%, from about 0.1% to about 2%, from about 0.2% to about 1.5%, from about 0.3% to about 1 % based on the total weight of the triglycerides. In some embodiments, the amount of *Thermomyces lanuginosus* can
20 be about 0.0001, 0.0002, 0.0005, 0.0007, 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.075, 0.10, 0.25, 0.50, 0.75, 0.80, 0.85, 0.90, 0.95, 1.0, 1,1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3,1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0% of the total weight of the triglycerides.

25 [75] The conditions for hydrolysis of the triglycerides may be routinely chosen by a person skilled in the art, having regard to the desired extent of hydrolysis and the specific triglyceride composition to be partially hydrolysed. Variables that may be controlled include the reaction time, the reaction temperature, the pH, the lipase concentration and agitation of the contact mixture comprising the triglycerides and the
30 aqueous phase. In general, longer reaction times, higher temperatures (within the operable limits of the lipase), higher amounts/concentrations of lipase and more turbulent agitation will favour a more complete extent of hydrolysis. A person skilled

in the art may be assisted to select appropriate conditions by monitoring the reaction with known analytical techniques.

[76] In some embodiments, the aqueous solution of the lipase may have a pH between about 6 and about 9. In some embodiments, the pH is between about 7 and about 8, or between 7.0 and 8.0. In some embodiments, the pH may be between 7.2 and 7.5. In one embodiment, the pH is about 7.5. The aqueous solution of the lipase may be a buffered solution, for example a phosphate buffered solution.

[77] Partial hydrolysis of the triglycerides may be effected at an elevated temperature. Suitable temperatures may include, but are not limited to, from about ambient temperature to about 100°C, or from about 35°C to about 80°C, or from about 40°C to about 50°C. The required reaction time to achieve a targeted extent of hydrolysis may be adjusted by varying the temperature. Thus, reaction times can vary from about 1 hour to about 72 hours or more, or from about 2 hours to about 72 hours, or from about 2 hours to about 24 hours, or from about 2 hours to about 15 hours.

[78] Typically, the partial hydrolysis reaction is conducted in the absence of an alcohol. The hydrolysis reaction therefore produces free fatty acids liberated from the glycerides in the form of protonated fatty acids (i.e. RCOOH), and not fatty acid esters. However, it is not excluded that alcohols, or other compounds comprising an acid-reactive functionality, are present in the reaction mixture. As used herein, the term “free fatty acid” may therefore include fatty acids liberated from glycerides in any form, including as esters of an alcohol present in the reaction mixture.

[79] In some embodiments, the contact between the triglycerides and the aqueous phase comprising the lipase is performed under an inert or substantially inert atmosphere, such as under a nitrogen or argon atmosphere which excludes or substantially excludes oxygen (O_2). This may prevent or reduce undesired oxidation of the glycerides or free fatty acids in the mixture.

[80] In one embodiment, the triglycerides / aqueous lipase mixture is agitated for a period of from about 2 hours to about 24 hours, such as 3 hours or 13 hours, at about 40°C under a nitrogen atmosphere.

[81] After partial hydrolysis, the partially hydrolysed triglycerides (comprising a glyceride fraction, a free fatty acid fraction and, in some embodiments, glycerol) can optionally be washed with water one or more times. The aqueous portion of the mixture can then be separated from the non-aqueous portion, and the non-aqueous
5 portion dried. A variety of drying methods and conditions disclosed in the art may be employed. In one embodiment, the non-aqueous portion is dried under vacuum at about 80°C.

Selective hydrolysis

[82] In some embodiments, the lipase selectively hydrolyses the triglycerides, preferentially hydrolysing undesired fatty acids such as shorter chain fatty acids and/or saturated fatty acids so that the concentration of desired longer chain omega-3 polyunsaturated fatty acids is increased in the glyceride composition. Such selectivity may be achieved through the use of an enzyme with either or both of positional specificity or fatty acid specificity.

[83] In many commercially important fish oils, certain desired omega-3 polyunsaturated fatty acids, particularly DHA, preferentially reside at the central position (sn-2) on the glyceride backbone. Therefore, the use of a lipase with a sn-1,3 hydrolysis specificity will increase the concentration of DHA in glycerides that are partially hydrolysed with that lipase. Furthermore, it has been reported that certain lipases, for example *Thermomyces lanuginosus* lipase, have desirable fatty acid specificities. *Thermomyces lanuginosus* is capable of preferentially hydrolysing shorter chain and saturated fatty acids such as mysteric acid (C14:0), palmitic acid (C16:0), palmitolenic acid (C16:1n7) and oleic acid (C18:1n9) from glycerides, relative to both EPA and DHA, through a fatty acid selectivity effect. DHA is particularly resistant to hydrolysis by *Thermomyces lanuginosus* lipase, relative to other fatty acids commonly found in fish oils, and this lipase is thus particularly useful for producing partially hydrolysed glycerides that are enriched in DHA.

[84] In some embodiments, the lipase is not a lipase of Candida rugosa (also known as Candida cylindracea), Geotrichum candidum, Mucor miehei, Penicillium roguefortii, or Pseudomonas fluorescens. Such enzymes, while capable of partially hydrolysing triglycerides, are not specific.

Separation

[85] Free fatty acids are then removed from the partially hydrolysed triglycerides. Either a portion of, substantially all, or all of the free fatty acids liberated from the triglycerides can be separated from the hydrolysed glyceride fraction. In one embodiment, substantially all of the free fatty acid fraction is separated from the glyceride fraction.

[86] Any suitable separation technique may be used to fractionate the hydrolysed glyceride and free saturated fatty acid fractions and the disclosed process is not intended to be limited to a particular fractionation technique or a specific set of fractionation conditions. One of skill in the art could readily select an appropriate fractionation technique to separate at least a portion of the hydrolysed glyceride fraction from the saturated free fatty acid fraction in the hydrolysed glyceride composition.

[87] In one embodiment, the free fatty acids are removed by molecular distillation, for example using wiped film evaporators and/or short path distillation equipment. Since environmental pollutants such as pesticides and polychlorinated biphenyls (PCBs) are more volatile than glycerides of long chain fatty acids, molecular distillation will remove these compounds, if present, from the glyceride fraction. This is another advantage of the use of molecular distillation in the present process.

[88] In another set of embodiments, the free fatty acids are separated from the partially hydrolysed glycerides by solvent extraction methods. In one embodiment, the free fatty acids are neutralised with a base, such as KOH. The amount of base to be added may be calculated based on a theoretical or measured acid value in the mixture. Alternatively, base may be added until the pH of the mixture exceeds a chosen value, for example 10, which is taken as an indicator that the fatty acids have been completely neutralised. The partially hydrolysed glycerides are then extracted into organic solvents and separated from the aqueous phase comprising the neutralised fatty acids. Several extractions of the aqueous phase may be performed to maximise the recovered yield of glycerides. Various organic solvents may be used for the extractions, including diethyl ether and hexane. The organic phase may then optionally be washed with an aqueous phase and/or dried to remove water. The glycerides are recovered from the organic phase by removal of the organic solvents, for example under vacuum.

Further treatment of the glycerides before microencapsulation

[89] In some embodiments, partial hydrolysis as described herein may be a substitute for a winterization process. Winterization is the process of removing high melting fractions (typically saturated fatty components) that solidify and cloud certain

natural oils (e.g. algal oils and fish oils), leaving an oil that remains clear and free of particulate. In a typical winterization process, the oil is cooled (e.g. to below minus 2°C) in order to crystallise out high melting point components. The crystals are then removed, for example by centrifugation or filtration with a filter press. Partial
5 hydrolysis of triglycerides by a lipase, particularly selective partial hydrolysis that preferentially removes saturated fatty acids, may thus eliminate the need for a winterization step.

[90] In some embodiments, glycerides comprising at least one of monoglycerides and diglycerides may be combined with triglycerides to provide the
10 glycerides to be microencapsulated. For example, in some embodiments, partially hydrolysed triglycerides, from which free fatty acids have been removed, may be combined with non-hydrolysed triglycerides to provide the glycerides to be microencapsulated. In these embodiments, the non-hydrolysed triglycerides may be the same as or different to the triglycerides that were partially hydrolysed. A person
15 skilled in the art would readily be able to determine the ratios of partially hydrolysed triglycerides and non-hydrolysed triglycerides to combine, having regard to the extent of hydrolysis in the partially hydrolysed triglycerides and the desired amount of monoglycerides and/or diglycerides in the composition to be microencapsulated.

[91] In some embodiments, partially hydrolysed triglycerides, from which free
20 fatty acids have been removed, are partially re-esterified with a desired fatty acid (or fatty acid ester), such as an omega-3 polyunsaturated fatty acid (or ester thereof). This will advantageously increase the content of omega-3 polyunsaturated fatty acids in the microencapsulated oil. Nevertheless, re-esterification must not be allowed to proceed to completion, since the glycerides to be microencapsulated must comprise
25 at least one of monoglycerides and diglycerides. A person skilled in the art, with the benefit of this disclosure, would be able to select an appropriate extent of re-esterification by balancing the competing imperatives to maximise omega-3 polyunsaturated fatty acid content, while retaining sufficient monoglycerides and/or diglycerides in the composition. Partial re-esterification, if performed, may be
30 performed through any suitable technique. In one embodiment, partial re-esterification is effected by contacting the partially hydrolysed glycerides with free fatty acids under anhydrous conditions in the presence of a suitable enzyme, such as

a lipase. A non-limiting example of a suitable lipase for re-esterification is Candida antarctica lipase B (CALB).

[92] In some embodiments, antioxidants may be added to the glycerides to be encapsulated. The use of antioxidants is one of the most common methods to prevent fish oil oxidation, and may provide further stabilisation to the microencapsulated glyceride composition beyond the stabilisation provided by the microencapsulation itself. Many different antioxidants have been utilised to stabilise fish oils, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Ethylenediaminetetraacetic acid (EDTA), tocopherols, ascorbic acid, ascorbyl palmitate, propyl gallate, gallic acid and lactoferrins. In one embodiment, Duralox® AN-110 XT antioxidant blend is used. Alternatively, natural antioxidants instead of synthetic compounds may be used, including plant extracts such as from oregano, rosemary, or parsley.

Preparation of the mixture comprising an emulsion and one or more polymer components

[93] Once the glycerides comprising at least one of monoglycerides and diglycerides and comprising at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides have been provided, a mixture is then prepared, comprising an emulsion wherein droplets of the glycerides are dispersed in an aqueous continuous phase, the mixture further comprising one or more polymer components.

[94] An emulsion is a fluid colloidal system comprising at least two immiscible liquid phases. In an emulsion, one liquid (the dispersed phase) is dispersed as droplets in the other liquid (the continuous phase). Commonly, one of the liquid phases is aqueous and the other is an oil. Emulsions with droplets of the oil phase dispersed in the aqueous phase are known as oil-in-water (O/W) emulsions. Typically, an emulsifier is required to stabilise an emulsion. Emulsifiers are often surface active agents (surfactants), which interact with both the oil phase and the water phase, thus accumulating at and stabilising the oil-water interface.

[95] The oil phase of the emulsion comprises the glycerides comprising at least one of monoglycerides and diglycerides, and at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides. In one embodiment, the oil phase consists of, or consists essentially of these glycerides. In
5 another embodiment, the oil phase comprises these glycerides and other, non-glyceride edible oils. The glycerides may already be a liquid at ambient conditions. However, it may be necessary to liquefy the glycerides to form an oil phase by heating, either prior to combination with the aqueous phase or when already in contact with the aqueous phase.

10 [96] The aqueous continuous phase comprises water, which in some embodiments is high purity water such as Milli-Q water (produced by Merck Millipore). In some embodiments, the pH of the aqueous phase is above 6, or above 7, or above 8.

[97] The mixture comprising an emulsion of droplets of the glycerides dispersed
15 in an aqueous continuous phase further comprises one or more polymer components, for encapsulating the droplets in the subsequent microencapsulation step. In some embodiments, at least one of the one or more polymer components is a biopolymer or a synthetically modified biopolymer. Suitable biopolymers may include polypeptides, such as proteins, and polysaccharides. In some embodiments, each of the one or
20 more polymer components is a biopolymer or a synthetically modified biopolymer.

[98] The one or more polymer components are typically water-soluble polymers. In some embodiments, at least one polymer component has surface active properties, so that it is capable of stabilising an O/W emulsion of the glycerides in the aqueous phase. This property may be an important, and limiting, consideration when
25 selecting the one or more polymer components for microencapsulation. However, as will be appreciated by a person skilled in the art, other considerations are also important, including the suitability of the polymer(s) for the subsequent microencapsulation method and ultimately the ability of the polymer system to stabilise the glycerides against oxidation by forming an effective oxygen barrier
30 around the droplets. The polymer system must be edible, must release the microencapsulated oil when ingested, and preferably have a bland taste. A wide

variety of different polymer systems for microencapsulating emulsified oils have been reported, and are available to a person skilled in the art.

[99] In some embodiments, at least one of the one or more polymer components is dissolved in the aqueous continuous phase, and in some embodiments, each of the one or more polymer components is dissolved in the aqueous continuous phase. Typically dissolution of the polymer components in the aqueous phase will be effected prior to combining the aqueous phase with the glycerides. For some polymer systems, it is necessary to dissolve a polymer component in the aqueous phase prior to emulsification to rehydrate the polymer component. In some embodiments, heating is required to fully effect the rehydration.

[100] The glycerides, the aqueous phase and the one or more polymer components may be combined in any suitable order. In some embodiments, the aqueous phase comprising at least one polymer component is combined with the glycerides, an emulsion is formed, and at least another polymer component is then added to the emulsified mixture, for example as an aqueous solution or dispersion. In other embodiments, the aqueous phase comprising each of the polymer components is combined with the glycerides, and an emulsion is subsequently formed.

[101] The glyceride oil phase and the aqueous phase may be emulsified by any suitable means. Typically, the glycerides and the aqueous phase are emulsified in the presence of at least one polymer component, which acts as an emulsifier. In some embodiments, a coarse emulsion is first prepared, by high shear mixing with a mechanical stirrer or a colloid mill. A final emulsion is then prepared by homogenisation, for example by high pressure microfluidisation or ultrasonic emulsification. In general, finer emulsions, i.e. those with smaller droplets sizes of the oil phase, will result in improved microencapsulation. In some embodiments, the droplets may have an average diameter of less than 100 µm, or less than 50 µm, or less than 25 µm, or less than 10 µm. The droplets may have an average diameter of from 40 nm to 10µm, or from 100 nm to 5µm, or from 0.1 µm to 2µm, or of about 1 µm. Droplet size may be measured using any typical equipment known in the art, for example, a Coulter™ LS230 Particle Size Analyzer, Miami, Florida, USA.

[102] Without wishing to be bound by any theory, it is believed that glycerides comprising at least one of monoglycerides and diglycerides form finer and/or more stable emulsions than their corresponding triglycerides. The monoglyceride and diglyceride components may accumulate at the oil-water interface, cooperating with
5 the one or more surface active polymer components to form an improved emulsifier system.

[103] A person skilled in the art will appreciate that several other parameters may be optimised by routine experimentation when forming the emulsified mixture comprising the glycerides, the aqueous phase and the one or more polymer
10 components. These include the concentration of polymer components in the aqueous phase, the viscosity of the aqueous phase, and the ratio (w/w) of oil phase to polymer components (known as the core-to-wall ratio). Higher polymer concentrations, viscosities and lower core-to-wall ratios will tend to increase the effectiveness of microencapsulation. However, the polymer concentration in the aqueous phase is
15 typically limited by either or both of the polymer solubility and maximum viscosity limitations. Highly viscous mixtures may be difficult to emulsify and to subsequently process into microcapsules. The optimum core-to-wall ratio may in practice be determined by trading off the improved stabilisation achieved at high relative polymer content against the imperative to maximise loading of glycerides in the
20 microencapsulated composition.

[104] One or more further additives may also be added to the mixture, either before or after emulsification. These may include non-polymeric emulsifiers, anti-oxidants, viscosifiers or agglomeration additives to promote agglomeration of primary microcapsules, once formed.

25 ***Polymer components for microencapsulation by spray-drying***

[105] Suitable polymer systems for microencapsulating droplets of the glycerides by spray-drying may include any polymer systems conventionally used for production of edible spray-dried oil-containing microcapsules. In some embodiments, carbohydrates such as hydrophobically modified starches are used. Starches such
30 as corn, barley and wheat starches may be hydrophobically modified in a variety of ways, including by succinylation. Hydrophobically modified carbohydrates offer

excellent emulsification capabilities. Examples of suitable hydrophobically modified starches include commercially available products such as Capsul®, N-lok® and Hi-Cap® starches, produced by Ingredion Inc. Cyclodextrins, also produced from starch, may also be suitable as polymer components for spray during encapsulation. 5 Partially hydrolysed starches, such as maltodextrins, offer excellent oxygen-barrier properties, but are typically poor emulsifiers, and may therefore be used in combination with other polymer components that are better capable of effectively stabilising emulsions.

[106] In some embodiments, at least one polymer component in the mixture for 10 spray-drying microencapsulation is a protein. Suitable proteins include gelatin, soy protein, and milk proteins such as whey protein concentrate, skimmed milk powder and caseinates. In some embodiments, the one or more polymer components comprise, or consist of, gelatin. When used in spray-drying applications, careful control of the pH must be exercised, as proteins may lose their emulsification 15 properties if the pH approaches the isoelectric point of the protein, leading to a loss of emulsion stability.

[107] In some embodiments, natural gums such as gum acacia (also known as gum arabic), mesquite gum and guar gum are used as a polymer component. A variety of other biopolymers have also been disclosed as suitable encapsulants for 20 spray-dried emulsions, including modified cellulose, alginates, chitosan, soluble soy polysaccharides and Maillard reaction products.

[108] In some embodiments where spay-drying microencapsulation is performed, the core-to-wall ratio in the emulsified mixture is from 0.05:1 to 5:1, or from 0.1:1, or from 0.15:0.5. In some embodiments, the core-to-wall ratio in the emulsified mixture 25 is from 0.2 to 0.3. Higher core-to wall ratios advantageously lead to higher glyceride loading in the microencapsulated composition, but in practice the core-to-wall ratio is limited by one or more of poor encapsulation yield of the glycerides, reduced encapsulation stabilisation and elevated surface oil content in the microcapsules at higher core-to-oil ratios.

30 [109] In some embodiments where spay-drying microencapsulation is performed, the polymer component composition in the aqueous phase will be greater than about

5%, or 10%, or 15%, or 20%. Higher polymer concentrations are advantageous, as less water must be evaporated in the subsequent spray-drying step, and a semi-permeable crust of polymer encapsulant will form sooner around the droplets during spray-drying, leading to improved encapsulation efficiency. However, the polymer component concentration in the aqueous phase will be limited by one or more of the polymer solubility or the viscosity of the aqueous phase.

Polymer components for microencapsulation by coacervation

[110] Suitable polymer systems for microencapsulating droplets of the glycerides in polymer coacervates include any polymer systems conventionally used for production of edible oil-containing coacervate microcapsules from an oil-in-water emulsion. In some embodiments, polymer components may be selected based on their ability to form simple coacervates: simple coacervation has been reported as a means to microencapsulate various natural oils. For simple coacervation, the one or more polymer components may consist of a single polymer component, which in some embodiments is an animal-derived protein such as gelatin or a plant-derived protein such as gliadin. In other embodiments, the single polymer component is a carbohydrate such as modified cellulose (for example, ethyl cellulose or hydroxypropyl cellulose).

[111] In some embodiments, the one or more polymer components comprise polymer components that may be induced to form a complex coacervate. Many suitable polymer systems for microencapsulating oils with a complex coacervate have been reported, and the droplets of glycerides may be microencapsulated according to the present invention with any of these. In some embodiments, the one or more polymer components comprise a protein polymer component and a polyanionic polymer component.

[112] Typically, the protein polymer component has an isoelectric point when dissolved in an aqueous phase. The isoelectric point is the pH above which a polymer carries a net negative charge, and below which the polymer carries a net positive charge. Furthermore, suitable protein polymer components are typically emulsifying agents, capable of stabilising an emulsion of droplets of glycerides dispersed in the aqueous phase when the pH of the aqueous phase is above the

isoelectric point of the protein. Suitable protein polymer components may include, but are not limited to, gelatin, β-lactoglobulin, bovine serum albumin, egg albumin, caseinate, soy proteins, pea proteins, whey protein and mixtures thereof. In some embodiments, the protein polymer component is selected from the group consisting of gelatin and whey protein isolate. In some embodiments, the protein polymer component is gelatin. In some embodiments, the protein polymer component is gelatin type A. In some embodiments, the gelatin type A has a Bloom strength of from 50 to 350, or between 200 and 300.

[113] As used herein, a polyanionic polymer component is a polymer component that, when dissolved in the aqueous phase, bears a net negative charge throughout the microencapsulation process. It is not excluded that a polyanionic polymer, when dissolved in an aqueous phase, may have an isoelectric point. However, in this case, the pH of the aqueous phase when forming the coacervates will be maintained above the isoelectric point of the polyanionic polymer. If the polyanionic polymer component has an isoelectic point, it will be lower than that of the protein polymer component with which it is paired for complex coacervate formation.

[114] Suitable polyanionic polymer components include, but are not limited to, gum arabic, pectin, chitosan, gelatin type B, carageenan, alginate, carboxymethylcellulose and polyphosphates (including sodium hexametaphosphate). In some embodiments, the polyanionic polymer component is selected from the group consisting of gum arabic and polyphosphates. In some embodiments, the polyanionic polymer component is a polyphosphate, such as sodium hexametaphosphate.

[115] In some embodiments, an aqueous solution of the protein polymer component is prepared, the glycerides and the aqueous protein solution are emulsified, and the polyanionic polymer component is subsequently added as an aqueous solution to the emulsion. However, it is not excluded that the aqueous phase comprises both the protein polymer component and the polyanionic polymer component when the emulsion is formed.

[116] The optimum ratio of the protein polymer component to the polyanionic polymer component will depend on the polymer system selected for microencapsulation, and may be determined by measuring the coacervate yield when

the two components are coacervated from an aqueous solution. For example, when the protein polymer component is gelatin type A, and the polyanionic polymer component is sodium hexametaphosphate (SHMP), the ratio (w/w) of gelatin:SHMP may be from about 7.5:1 to about 35:1, or from about 10:1 to about 30:1, or about 15:1. By contrast, when the protein polymer component is whey protein isolate (WPI), and the polyanionic polymer component is gum arabic (GA), the ratio (w/w) of WPI:GA may be from about 1:1 to about 5:1, or from about 2:1 to about 4:1, or about 3:1.

[117] In embodiments where microencapsulation by coacervation is performed, the amount of the coacervate-forming polymer component or components provided in the emulsified mixture should be sufficient to form the primary shells encapsulating the droplets of glycerides, and optionally also any secondary shells that are to be formed around agglomerations of the primary shells. In some embodiments, the polymer component or components are provided in a combined amount of from about 1% to about 15% by weight of the aqueous phase, or from about 2% to about 8% by weight, or from about 3% to 5% by weight.

Microencapsulation

[118] Once the mixture comprising the emulsion and the one or more polymer components has been prepared, the droplets of the glycerides are microencapsulated with the one or more polymer components by a method selected from spray-drying the mixture and inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets. As used herein, microencapsulation means a process in which the oil-phase droplets are surrounded by a solidified coating to give small capsules (i.e. microcapsules). These microcapsules, in the absence of the aqueous phase, typically form a free flowing dry powder.

[119] Microencapsulation by spray-drying and by coacervate formation both require a polymer-stabilised emulsion of the oil phase to be encapsulated dispersed in an aqueous phase. In both techniques, the size and stability of the precursor emulsion is an important variable which affects the stability of the microencapsulated oils.

Microencapsulation by spray-drying

[120] When using spray-drying as the method of microencapsulation, the emulsified mixture is transferred, for example by pumping, to the drying chamber of a spray-drier, where it is atomised to form drops of the emulsion. The atomised drops fall through the hot air medium in the drying chamber, where water rapidly evaporates, leaving particles in which droplets of glycerides are embedded in a matrix of the one or more polymer components. The spray-dried particles are typically only exposed to the high temperature conditions in the drying chamber for a few seconds, so that the glycerides embedded in the particles may not reach the external temperature in the drying chamber. The spray-dried microcapsules typically have a multi-core structure, in which multiple droplets of oil are embedded in each spray dried particle.

[121] Atomisation of the emulsified mixture may be performed by conventional methods, including with a high pressure nozzle or a centrifugal wheel. Several process parameters are routinely optimised by persons skilled in the art when developing a spray-drying microencapsulation process. The optimisation will depend on, for example, the composition of the emulsified mixture and the desired particle size. Particle size may be controlled by selecting the nozzle orifice size and pressure or the wheel diameter and speed, by controlling the emulsion viscosity and/or by selecting the spray-drying temperature. In some embodiments, the spray-dried particles have an average diameter below about 150 μm, or from about 1 μm to about 100 μm, or from about 10 to about 100 μm.

[122] High spray pressures, a wide spray pattern and higher air flows in the drying chamber may also improve microencapsulation effectiveness, as the improved mass transfer that results will lead to more rapid and more even drying and formation of a protective crust around the glycerides droplets. The air inlet temperature is another parameter that a person skilled in the art will routinely optimise for a given spray-drying application. Higher inlet air temperatures may accelerate drying and crust formation, thus improving microencapsulation effectiveness, although excessively high temperatures may lead to heat damage or ballooning of the particles due to steam pressure in the particle. In some embodiments, the air inlet temperature

may be from about 140°C to about 300°C, or from about 150°C to about 220°C, or from about 160°C to about 200°C, or from about 160°C to about 180°C.

Microencapsulation by simple coacervation

[123] When microencapsulating the glycerides with coacervates, the one or more polymer components in the emulsified mixture are induced to form a coacervate phase which forms primary shells encapsulating the droplets. The method of inducing coacervation will depend on the polymer system for microencapsulation.

[124] Simple coacervation may be induced by salting out the polymer component by adding an electrolyte, by adding a water miscible non-solvent for the polymer, or by decreasing the temperature of the emulsion. In some embodiments where a protein coacervate is the encapsulant (for example gelatin, gliadin or soy-protein isolate), simple coacervation may be induced by adjusting the pH towards the isoelectric point of the protein, or by adding an electrolyte such as sodium sulphate, or by adding a water-miscible non-solvent, such as ethanol. In some embodiments where a polysaccharide coacervate such as hydroxypropyl methylcellulose is the encapsulant, simple coacervation may be induced by adding another component, such as maltodextrin, which has a higher solubility in the aqueous phase than the encapsulant.

Microencapsulation by complex coacervation

[125] When microencapsulating the glycerides with complex coacervates, the emulsified mixture comprises at least two polymer components, and the polymer components are induced to form a coacervate phase which forms primary shells encapsulating the droplets. In some embodiments, the one or more polymer components comprise a protein polymer component and a polyanionic polymer component, and the droplets of the glycerides are microencapsulated by inducing complex coacervation of the protein polymer component and the polyanionic polymer component to form primary shells of complex coacervate encapsulating the droplets.

[126] Any suitable means of inducing the two or more polymer components to coacervate may be used. Complex coacervation may induced by adjusting pH, temperature, concentration, mixing speed or a combination thereof in the mixture. In some embodiments, complex coacervation is induced by adjusting pH in the mixture. In some embodiments, the pH is reduced by adding an acid. Suitable acids may

include, but are not limited to, phosphoric acid, citric acid, acetic acid. In some embodiments, complex coacervation is induced by adding a second polymer component to the emulsified mixture comprising a first polymer component, wherein the conditions, including pH conditions, of the combined mixture are such that
5 coacervation automatically commences when the two polymer components come into contact.

[127] In some embodiments where the one or more polymer components comprise a protein polymer component and a polyanionic polymer component, the pH is reduced to, or to below, the isoelectric point of the protein. The preferred pH may
10 be readily determined by a person skilled in the art for a given coacervation system, for example by measuring the turbidity of an aqueous mixture of the polymer components as the pH is reduced. Without wishing to be bound by any theory, it is believed that below the isoelectric point, the net charge on the protein polymer component becomes positive due to protonation of neutral amino groups to form
15 cationic ammonium groups and/or protonation of anionic carboxylate groups to form neutral carboxylic acid groups. The positively charged protein is then attracted to the negatively charged polyanionic polymer component, forming complexes which are no longer soluble in the aqueous phase. As a result, a complex coacervate forms as a second liquid phase.

20 [128] When complex coacervation is induced, the complex coacervate forms primary shells around the droplets of glycerides in the mixture. Without wishing to be bound by any theory, in some embodiments at least one polymer component, typically a protein polymer component, accumulates preferentially at the oil-water interface of the emulsion as an emulsifier, so that when coacervation is induced, the coacervate
25 forms at the oil water-interface as a shell surrounding the droplets.

[129] Complex coacervation may be induced at any suitable temperature. In some embodiments, the temperature is between about 5°C and about 80°C, or between 15°C and about 60^{0}C, or between about 20°C and 55°C.

Multicore coacervation

[130] In some embodiments, the primary shells of coacervate become the walls of the final microcapsule particles of the microencapsulated omega-3 polyunsaturated fatty acid glyceride composition. Such microcapsules are known as single-core microcapsules.

5 [131] In some embodiments, agglomerations of the primary shells form in the mixture, so that the final microcapsule particles of the microencapsulated omega-3 polyunsaturated fatty acid glyceride composition are characterised by a multi-core configuration. In some embodiments, a secondary shell of coacervate is formed around the agglomerations, so that the droplets of glycerides in the final microcapsule 10 particles are encapsulated within both the primary and the secondary shell. Such multi-shell microcapsules may provide enhanced protection of the encapsulated glycerides against oxidation.

[132] In some embodiments, droplets of the glycerides are microencapsulated by inducing complex coacervation of a protein polymer component and a polyanionic 15 polymer component to form primary shells of complex coacervate encapsulating the droplets, agglomerations of the primary shells are then produced, and the mixture is cooled to form secondary shells of complex coacervate encapsulating the agglomerations. Agglomerations of the primary shells may be produced by controlling the stirring rate, for example by reducing the stirring rate, or by adjusting the pH or by 20 cooling the mixture, or by combinations of these actions. Alternatively, the primary shells may agglomerate without intervention, and suitable agglomerations of the primary shells are produced by allowing sufficient time for agglomeration to occur. Agglomeration of the primary shells is typically produced above the gel point of the coacervate phase.

25 [133] Secondary shells of complex coacervate are formed around the agglomerations by cooling the mixture. Cooling of the mixture may cause dissolved or colloidal complexes of the oppositely charged polymer components to form additional coacervate material, which accumulates on the walls of the agglomerates to form the secondary shells. Alternatively, free coacervate suspended in the 30 aqueous phase may accumulate on the agglomerations. In some embodiments, the mixture is cooled by reducing the temperature at a rate of from about 1°C/h to about

20°C/h, or from about 5°C/h to about 15°C/h. In some embodiments, the mixture is cooled to below 15°C, or below 10°C, or from about 5°C to about 10°C.

[134] In some embodiments, additional polymer components may be added to the mixture comprising the agglomerations of primary shells, either of the same kind or a different kind to the components forming the primary shells, in order to thicken the secondary shells and/or to produce microcapsules having primary and secondary shells of different compositions. In some embodiments, a third shell may be formed by additional polymer components, as a further encapsulating layer over the secondary shells.

Cross-linking

[135] The microencapsulation process may include a step of cross-linking the one or more polymer components in the encapsulating matrix. Cross-linking the polymer components may solidify or harden the encapsulant, thus providing or improving the structural integrity of the microcapsules. Cross-linking the polymer components may also improve the oxygen barrier properties of the encapsulant.

[136] Cross-linking may be performed by any suitable method for the encapsulating polymer system. In some embodiments, cross-linking is achieved by a heat treatment, for example by heating the microcapsules to about 80°C. In some embodiments, heat-induced cross-linking is achieved when subsequently spray-drying the microcapsules. In some embodiments, a cross-linking agent is added to cross-link the one or more polymer components, typically while the microcapsules are still in the mixture in which they were formed. Both chemical and enzymatic cross-linking agents may be used. Examples of chemical cross-linking agents include aldehydes such as glutaraldehyde and formaldahyde. Examples of enzymatic cross-linking agents include transglutaminase.

Processing and properties of the coacervate-encapsulated microcapsules

[137] When microencapsulating the glycerides with coacervates, the microcapsules produced may be washed with water and/or dried to provide a free-flowing powder. Anti-oxidants may be added to the microcapsules before the drying step. Drying can be accomplished by a number of methods known in the art such as,

for example, freeze drying, drying with ethanol, or spray drying. In one embodiment, the microencapsulated omega-3 polyunsaturated fatty acid glyceride composition is dried by spray-drying or freeze-drying. Suitable spray drying techniques are available to a person skilled in the art. Drying agents or anticaking agents may be added to the 5 microcapsules to help produce free flowing powders.

[138] In some embodiments, the coacervate-encapsulated microcapsules have an average diameter of below about 150 μm, or from about 1 μm to about 100 μm, or from about 10 to about 100 μm.

Microencapsulated glyceride compositions

10 [139] The present invention also relates to a microencapsulated glyceride composition enriched in omega-3 polyunsaturated fatty acids, specifically comprising at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides. The microencapsulated glycerides comprise at least one of monoglycerides and diglycerides in a total amount of at least 5% based on the total 15 mass of the glycerides. Droplets of the glycerides are microencapsulated with an encapsulant which comprises one or more polymer components. The microencapsulated glyceride compositions may be formed by any of the processes described herein, using any of the materials described herein.

[140] In some embodiments, the encapsulant is a complex coacervate formed as 20 primary shells encapsulating the droplets. In some embodiments, the complex coacervate encapsulating the droplets comprises a protein polymer component and a polyanionic polymer component.

[141] In some embodiments, the microencapsulated glyceride composition comprises agglomerations of the primary shells, and the agglomerations are 25 encapsulated with secondary shells of complex coacervate.

Products and uses

[142] The present invention also relates to a nutritional supplement, functional food, medical food or infant formula comprising the microencapsulated omega-3

polyunsaturated fatty acid glyceride compositions according to any of the embodiments described herein.

[143] As used herein, a nutritional supplement is any composition that can be administered to or taken by a subject to provide, supply, or increase one or more nutrients (e.g. a vitamin, mineral, essential trace element, amino acid, peptide, nucleic acid, oligonucleotide, lipid, cholesterol, steroid, carbohydrate and the like).

[144] As used herein, a functional food is a food that contains known biologically-active compounds which when in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit.

[145] As used herein, a medical food is a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.

[146] The nutritional supplement, functional food, medical food or infant formula may comprise any amount of the microencapsulated glyceride compositions disclosed herein, but will typically contain an amount determined to supply a subject with a desired dose of an omega-3 poly-unsaturated fatty acid (e.g. EPA and/or DHA). The nutritional supplement, functional food, medical food or infant formula may further comprise other nutrients such as vitamins, trace elements, minerals, and the like.

[147] The present invention also relates to use of a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition according to any of the embodiments described herein, for the treatment or prevention of a disease or medical condition, wherein the disease or medical condition is treatable or preventable by ingestion of omega-3 polyunsaturated fatty acids.

EXAMPLES

[148] The present invention is described with reference to the following examples. It is to be understood that the examples are illustrative of and not limiting to the invention described herein.

Materials

[149] Anchovy oil and tuna oil were obtained from Nu-Mega Ingredients Pty Ltd (Melbourne, Victoria, Australia)

[150] Lipozyme® TL 100 L TL 100L was obtained from Novozymes Australia Pty.
5 Ltd.

[151] Antioxidant Duralox Blend AN 110 XT was obtained from Kalsec Incorporation (Kalamazoo, MI, USA).

[152] Gelatin (from porcine skin type A, 300 bloom) was purchased from Sigma–Aldrich Corporation (Sydney, NSW, Australia).

10 [153] Sodium hexametaphosphate was purchased from Sigma–Aldrich Corporation (Sydney, NSW, Australia).

[154] Transglutaminise (Activa® KS–LS) was purchased from Ajinomoto (Tokyo, Japan).

Example 1 – Partial hydrolysis and microencapsulation of anchovy oil

15 Partial hydrolysis of anchovy oil

[155] Lipozyme® TL 100L was mixed with 50 g anchovy oil in a dosage of 2,000 U/g oil. The mixture was flushed with nitrogen and incubated at 40°C with magnetic stirring at 300 rpm in phosphate buffer at a pH of 7.0, 7.5 and 8.0 for time periods of 12, 15.5 and 19 hours. The hydrolysis degree (HD) in the samples was determined
20 by capillary chromatography with flame ionisation detector (Iatroscan MK5, Iatron Laboratories Inc., Tokyo, Japan) using SIC-480 II software for multiple chromatogram processing, by comparing the percentage peak areas of the unhydrolysed and hydrolysed triglyceride (as reported in Akanbi et al, Food Chemistry, 2014, 160, 61-66).

25 [156] The effect of pH on the hydrolysis degree of anchovy oil is shown in Fig. 1, which shows that the highest activity of the lipase TL 100L was at pH 7.5.

[157] Lipozyme® TL 100L was mixed with 50 g anchovy oil in a dosage of 2,000 U/g oil. The mixture was flushed with nitrogen and incubated at 40°C with magnetic stirring at 300 rpm in phosphate buffer at a pH of 7.5 for time periods ranging from 2 to 15 hours. The HD values as a function of reaction time are shown in Figure 2.
5 Hydrolysis times of 3 and 13 hours were selected to prepare partially hydrolysed oil with 30 and 60% HD values.

[158] The glyceride and free fatty acid (FFA) fractions in the 30% and 60% HD hydrolysed oil were separated by solvent extraction. The theoretical amount (TA) of 0.5 M KOH solution in 30% ethanol (v/v) was calculated based on the acid value (AV)
10 of the hydrolysed oils. Then, 121% TA of this KOH solution was used to neutralise the FFA released during the hydrolysis. The incorporation of KOH was deemed complete once the pH of mixture exceeded 10. The glycerides were then recovered by extraction with diethyl ether, and then hexane, leaving the neutralised FFA in the aqueous phase. The organic solvents were then removed from the glycerides under
15 vacuum.

Analysis of the partially hydrolysed anchovy oils

[159] The lipid composition of the glycerides recovered from the partially hydrolysed oil with 30 and 60% HD values is shown in Table 1. Monoglycerides (MG) and diglycerides (DG), together with some residual FFA, were present in the partially
20 hydrolysed oils, while only triglycerides (TG) were detected in the native anchovy oil. MG and DG account for approximate 14 and 32% in both glycerides and their contents were irrespective of the HD values.

Table 1. Lipid composition of anchovy oil, 30 and 60% HD glycerides

	Lipid classes (weight % of recovered oil)			
	MG	DG	TG	FFA
Anchovy oil	0	0	100	0
30% HD glyceride	13.67	32.62	52.67	1.03
60% HD glyceride	13.96	31.01	51.93	2.14

[160] The fatty acid composition of anchovy oil, 30 and 60% HD glycerides is shown in Fig. 3. The EPA concentration in the glycerides was not substantially affected by the hydrolysis degree (15.6 and 15.9% for 30 and 60% HD glycerides, respectively, similar to the 16.6 % EPA in the native anchovy oil). However,
5 hydrolysis significantly increased the concentration of DHA, from 10.7% in the native anchovy oil to 16.3 and 20.3% in the 30 and 60% HD oil.

Oxidative stability of anchovy oil derived glycerides

[161] Duralox Blend AN 110 XT was incorporated into native anchovy oil and the 30% and 60% HD partially hydrolysed anchovy oil. The effect of antioxidant dosage
10 on the oxidative stability of these oils was studied using Rancimat at 70°C and an airflow rate of 10 L/h. As shown in Table 2, both partially hydrolysed glycerides exhibited decreased oxidative stability index (OSI) comparing to the native anchovy oil at the same antioxidant dosages, due to their higher concentration of unsaturated fatty acids. The incorporation of the antioxidant significantly increased the OSI of the
15 glycerides in the range of 0-0.4% (w/w) regardless of hydrolysis degree. Beyond this concentration, Duralox Blend AN 110 XT exhibited prooxidative activity for the partially hydrolysed oils, so the incorporation dosage of 0.4% (w/w) in the oil phase was used in subsequent experiments.

Table 2. Effect of antioxidant dosage on OSI of anchovy oil, 30 and 60% HD
20 glycerides

Antioxidant dosage (%, w/w)	OSI of the oil phase (hours)		
	Anchovy oil	30% HD glyceride	60% HD glyceride
0	5.38 ± 0.25	1.05 ± 0.05	0.75 ± 0.08
0.2	7.66 ± 0.23	1.11 ± 0.18	1.33 ± 0.06
0.4	12.53 ± 0.75	2.58 ± 0.16	2.28 ± 0.17
0.8	19.34 ± 0.18	1.83 ± 0.13	1.43 ± 0.09
1.2	32.71 ± 1.25	1.88 ± 0.29	1.02 ± 0.13

Microencapsulation of anchovy oil derived glycerides

[162] Samples of the native anchovy oil and the 30% and 60% HD partially hydrolysed anchovy oils (24 g) containing Duralox Blend AN 110 XT (0.4%, w/w) were mixed with 250 g (6%, w/w) of gelatin dispersion in water. This mixture was stirred at 1200 rpm for 5 min using a mechanical stirrer. An O/W emulsion was prepared by emulsifying the mixture at 15,000 rpm for 15 min using a homogenizer. Then, 250 g of sodium hexametaphosphate (SHMP) (0.4%, w/w in water) was poured into the emulsion and stirred at 600 rpm. The pH of the mixed emulsion was then adjusted to 4.7 by adding 1% phosphoric acid. The temperature in the coacervation process was controlled at 50°C until the coacervate had formed, followed by cooling to 5°C at a cooling rate of 6°C/h using a programmable refrigerated water bath (PolyScience Inc., Nioles, IL, USA). After maintaining the sample at 5°C for 30 min, 100 ml of 3% (w/w) transglutaminase dispersion was added to induce crosslinking. The slurry was heated to 25°C at a heating rate of 5°C/h to activate the enzyme and was kept at 25°C for 5 h to allow complete crosslinking. Finally, the microcapsules were freeze-dried (using an Alpha 1-4 LD plus freeze drier, Martin Christ GMBH, Osterode, Lower Saxony, Germany) to yield final powder products.

Analysis of the properties of the microencapsulated anchovy oil derived glycerides

[163] The morphology of the complex coacervate covered primary shells and final product microcapsules, as imaged by optical microscopy, is presented in Fig. 4. Coacervates formed in the aqueous phase induced the agglomeration of the O/W emulsion droplets (Fig. 4: A, C & E), resulting in the formation of multi-core microcapsules (Fig. 4: B, D & F). The partially hydrolysed glycerides produced emulsions with a smaller droplet size compared to emulsions prepared with the native anchovy oil, even though the emulsions were otherwise prepared in the same manner. The partially hydrolysed oils gave rod-shaped microcapsules, rather than the olive-shaped ones fabricated from the native anchovy oil. This is also evident from SEM images of the microcapsules, as shown in Fig. 5, which further shows that microcapsules prepared from partially hydrolysed glycerides exhibit smoother surfaces than the ones produced from native anchovy oil, possibly due to the decrease of O/W emulsion droplet size.

[164] The encapsulation properties of the freeze-dried microcapsules prepared with native anchovy oil and the 30% and 60% partially hydrolysed glycerides, including surface oil content, encapsulation efficiency, oil payload and encapsulation yield of the final microcapsules, are presented in Table 3. High payload (>50%) and 5 extremely high encapsulation efficiency (>97%) of the microcapsules was observed for all the oils.

[165] The physical properties of the microcapsules are also presented in Table 3. The size distributions of the microcapsules fabricated from native anchovy oil and the 30% and 60% partially hydrolysed glycerides were studied using a Malvern master 10 sizer. The size of all the microcapsules was found to be below 100 μm, low enough to avoid a change of food texture and mouth feel. The surface morphology of the fabricated microcapsules was investigated using AFM. The roughness values Ra of the partially hydrolysed glyceride-containing microcapsules were nearly 1/5 of those of the native anchovy oil-containing microcapsules.

15 Table 3. Encapsulation and physical properties of microcapsules

	Native anchovy oil microcapsule	30% HD glyceride microcapsule	60% HD glyceride microcapsule
Encapsulation properties			
Surface oil content (%)	1.01 ± 0.48	1.84 ± 0.60	1.81 ± 0.70
Payload (%)	53.07 ± 1.30	52.92 ± 2.41	50.80 ± 2.01
Encapsulation efficiency (%)	98.51 ± 0.51	97.07 ± 0.70	97.03 ± 0.82
Encapsulation yield (%)	98.15 ± 2.10	96.83 ± 1.25	90.93 ± 3.61
Microcapsule properties			
d (3, 2) (μm)	60.24 ± 7.45	28.92 ± 2.21	37.12 ± 0.89
d (4, 3) (μm)	86.78 ± 5.97	47.20 ± 5.34	61.21 ± 5.26
Span	1.63 ± 0.16	1.83 ± 0.07	1.77 ± 0.32
Surface roughness	482	114	80

Average elastic modulus (MPa)	4111.61	18220.20	20799.30

Oxidative stability of the microencapsulated anchovy oil derived glycerides

[166] The oxidative stability of the un-encapsulated glycerides (native anchovy oil, 30% and 60% partially hydrolysed glycerides, with 0.4% antioxidant), and the microcapsules containing these glycerides, were compared using Rancimat at 70°C at the airflow rate of 10 L/h. The results are shown in Fig. 6. The oxidative stability of the non-encapsulated partially hydrolysed glycerides was significantly lower than that of the native oil (Fig. 6: A), which is expected due to the higher unsaturated fatty acids concentration and/or the more exposed nature of the fatty acids. Furthermore, the oxidative stability of both the native and the partially hydrolysed anchovy oils were significantly enhanced due to microencapsulation, indicating that the shell material acts as an effective protection against oxygen.

[167] Surprisingly, as seen in Fig. 6: B, the microencapsulated, partially hydrolysed glycerides (which comprise monoglycerides and diglycerides) exhibited significantly higher oxidative stability than the microencapsulated, native oil microcapsules (which comprise only triglycerides). This is opposite to the relative stabilities of the un-encapsulated oils.

Example 2 – Microencapsulation of mixtures of partially hydrolysed anchovy oil and native anchovy oil

Preparation of mixtures of 30% HD glycerides with native anchovy oil

[168] The 30% HD partially hydrolysed glyceride, prepared as described in example 1, was mixed with native anchovy oil at various ratios. The 30% HD glyceride content in the final mixed oil compositions was 0, 3.13, 6.25, 12.5, 25, 50, and 100% (w/w). The compositions of the oils, as calculated from the ratios and the known compositions of the native anchovy oil and the 30% HD glyceride, are shown in Table 4.

Table 4. Lipid composition of mixtures of anchovy oil with 30 % HD glyceride

	Lipid classes (weight % of mixture)				
	MG	DG	TG	FFA	(MG+DG)/G*
Anchovy oil	0	0	100	0	0
3.13% of 30% HD	0.45	1.08	98.44	0.03	1.54
6.25% of 30% HD	0.85	2.04	97.04	0.06	2.92
12.5% of 30% HD	1.71	4.08	94.08	0.13	5.85
25% of 30% HD	3.42	8.16	88.17	0.26	11.69
50% of 30% HD	6.84	16.31	76.34	0.52	23.39
100% of 30% HD	13.67	32.62	52.67	1.03	46.78

*(MG+DG)/G = weight % of (monoglycerides + diglycerides) of total glycerides

Properties of the microencapsulated mixtures of 30% HD glycerides and native anchovy oil

5 [169] The mixtures, as shown in Table 4, were combined with Duralox Blend AN 110 XT at 0.4% dosage (w/w), and then microencapsulated by the same procedure as described in example 1. The oxidative stability index values were measured as discussed in example 1. The encapsulation properties and the oxidative stability properties of the resulting microcapsules are presented in Table 5.

10

Table 5. Encapsulation and oxidative stability properties of the microencapsulated mixtures

(MG+DG)/G*	Payload (%)	Encapsulation yield (%)	OSI (hours)
0	54.83 ± 1.17	98.24 ± 2.10	16.12 ± 0.22
1.54	53.02 ± 0.84	95.00 ± 1.50	19.07 ± 0.49
2.92	53.92 ± 0.57	96.62 ± 1.02	24.28 ± 1.11
5.85	53.21 ± 0.94	95.33 ± 1.69	30.09 ± 0.62
11.69	52.42 ± 0.96	93.91 ± 1.73	41.61 ± 0.46
23.39	53.10 ± 1.16	95.13 ± 2.09	45.03 ± 1.36
46.78	53.34 ± 0.81	95.56 ± 1.46	66.04 ± 1.02

*(MG+DG)/G = weight % of (monoglycerides + diglycerides) of total glycerides

[170] The oxidative stability of the compositions increased in proportion with the amount of the 30% hydrolysed oil in the mixture, despite the fact that the intrinsic oxidative stability of the oil phase, by itself, is expected to decrease with increasing degree of hydrolysis. By preparing a composition with just 5.85% of monoglycerides and diglycerides (out of the total glyceride content of the composition), the OSI could be nearly doubled.

Example 3 – Partial hydrolysis and microencapsulation of tuna oil

Preparation of microencapsulated, partially hydrolysed tuna oils

[171] Tuna oil was hydrolysed at a pH of 7.5, following the procedure of example 1. The HD values as a function of reaction time are shown in Figure 7. Hydrolysis degrees of 30 and 60% could be achieved after hydrolysis for 6 and 24 h, respectively, indicating that the hydrolysis of tuna oil is not as rapid as that of anchovy oil, possibly due to the different fatty acid composition. Glycerides with 30% and 60% HD values were recovered after the FFA were removed, following the method of example 1. The lipid composition of the partially hydrolysed glycerides is shown in Table 6.

Table 6. Lipid composition of tuna oil, 30 and 60% HD glycerides

	Lipid classes (weight%)		
	MG	DG	TG
Anchovy oil	0	0	100
30% HD glyceride	9.39	26.74	63.82
60% HD glyceride	11.19	29.24	59.57

[172] The major fatty acid composition of tuna oil, 30 and 60% HD glycerides is
5 shown in Fig. 8. The DHA concentration in the glycerides was significantly increased by the hydrolysis, from 24.84% in the native tuna oil to 36.29% and 44.50% for 30 and 60% HD glycerides, respectively.

[173] The native tuna oil, and the 30% and 60% HD partially hydrolysed tuna oils were combined with Duralox Blend AN 110 XT at 0.4% dosage (w/w), and then
10 microencapsulated by the same procedure as described in example 1.

Oxidative stability of the microencapsulated tuna oil derived glycerides

[174] The oxidative stability of the un-encapsulated glycerides (native tuna oil, 30% and 60% partially hydrolysed glycerides, with 0.4% antioxidant), and the microcapsules containing these glycerides, were compared using Rancimat at 90°C
15 at an airflow rate of 10 L/h. The results are shown in Fig. 9. The 30% and 60% HD glycerides exhibited large decreases in OSI values comparing to the native tuna oil, as would be expected. By contrast, enhanced OSI values were observed for both microencapsulated, partially hydrolysed glyceride compositions relative to the microencapsulated native tuna oil composition.

20 ***Example 4 – Microencapsulation of mixtures of partially hydrolysed tuna oil and native anchovy oil***

[175] Mixtures of the 30% HD tuna oil and the native tuna oil were prepared according to the procedure described in example 2. The compositions of the oils, as

calculated from the ratios and the known compositions of the native tuna oil and the 30% HD glycerides, are shown in Table 7.

Table 7. Lipid composition of mixtures of tuna oil with 30 % HD glycerides

	Lipid classes (weight % of mixture)			
	MG	DG	TG	(MG+DG)/G*
Tuna oil	0	0	100	0
3.13% of 30% HD	0.31	0.88	98.81	1.19
6.25% of 30% HD	0.59	1.67	97.74	2.26
12.5% of 30% HD	1.17	3.34	95.48	4.52
25% of 30% HD	2.35	6.69	90.96	9.03
50% of 30% HD	4.70	13.37	81.91	18.07
100% of 30% HD	9.39	26.74	63.82	36.15

*(MG+DG)/G = weight % of (monoglycerides + diglycerides) of total glycerides

5 [176] The mixtures, as shown in Table 7, were combined with Duralox Blend AN 110 XT at 0.4% dosage (w/w), and then microencapsulated by the same procedure as described in example 1. The oxidative stability index was measured as discussed in example 1, and the results are presented in Figure 10.

[177] The oxidative stability of the compositions increased in proportion with the 10 amount of the 30% hydrolysed oil in the mixture, despite the fact that the intrinsic oxidative stability of the oil phase, by itself, is expected to decrease with increasing degree of hydrolysis.

Claims

1. A process for preparing a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, the process comprising:
providing glycerides comprising at least one of monoglycerides and diglycerides in a total amount of at least 5% based on the total mass of the glycerides, wherein the glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides;
preparing a mixture comprising an emulsion wherein droplets of the glycerides are dispersed in an aqueous continuous phase, the mixture further comprising one or more polymer components; and
microencapsulating the droplets of the glycerides with the one or more polymer components by a method selected from spray-drying the mixture and inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets.

2. The process of claim 1, wherein the glycerides comprise at least one of monoglycerides and diglycerides in a total amount of at least 10% based on the total mass of the glycerides.

3. The process of claim 1 or claim 2, wherein the glycerides are provided by:
contacting triglycerides with a lipase under aqueous conditions for a time and at a temperature sufficient to partially hydrolyse the triglycerides to form partially hydrolysed triglycerides; and
removing free fatty acids from the partially hydrolysed triglycerides.

4. A process for preparing a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, the process comprising:
contacting triglycerides with a lipase under aqueous conditions for a time and at a temperature sufficient to partially hydrolyse the triglycerides to form partially hydrolysed triglycerides;
removing free fatty acids from the partially hydrolysed triglycerides to provide glycerides comprising at least one of monoglycerides and diglycerides,

wherein the glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on the total mass of glycerides;

preparing a mixture comprising an emulsion wherein droplets of the glycerides are dispersed in an aqueous continuous phase, the mixture further comprising one or more polymer components; and

microencapsulating the droplets of the glycerides with the one or more polymer components by a method selected from spray-drying the mixture and inducing coacervation of the one or more polymer components in the mixture to form primary shells of coacervate encapsulating the droplets.

5. The process of claim 3 or claim 4, wherein the lipase is *Thermomyces lanuginosus* lipase.

6. The process of any one of claims 3 to 5, further comprising combining the partially hydrolysed triglycerides with non-hydrolysed triglycerides to provide the glycerides.

7. The process of any one of claims 3 to 6, wherein the triglycerides are selected from the group consisting of fish oils, microbial oils, algal oils and combinations thereof.

8. The process of any one of claims 3 to 7, wherein the triglycerides are fish oil selected from the group consisting tuna oil, anchovy oil and combinations thereof.

9. The process of any one of claims 1 to 8, wherein the one or more polymer components comprise a protein polymer component and a polyanionic polymer component, and wherein the droplets of the glycerides are microencapsulated by inducing complex coacervation of the protein polymer component and the polyanionic polymer component to form primary shells of complex coacervate encapsulating the droplets.

10. The process of claim 9, further comprising:

producing agglomerations of the primary shells; and

cooling the mixture to form secondary shells of complex coacervate encapsulating the agglomerations.

11. The process of claims 9 or claim 10, wherein complex coacervation is induced by adjusting pH, temperature, concentration, mixing speed or a combination thereof in the mixture.

12. The process of any one of claims 9 to 11, wherein complex coacervation is induced by adjusting pH in the mixture.

13. The process of any one of claims 9 to 12, wherein the protein polymer component is selected from the group consisting of gelatin, β-lactoglobulin, bovine serum albumin, egg albumin, caseinate, soy proteins, pea proteins, whey protein and mixtures thereof, and is preferably gelatin, most preferably gelatin type A.

14. The process of any one of claims 9 to 13, wherein the polyanionic polymer component is selected from the group consisting of gum arabic, pectin, chitosan, gelatin type B, carageenan, alginate, carboxymethylcellulose and polyphosphates, and is preferably a polyphosphate, most preferably sodium hexametaphosphate.

15. The process of any one of claims 9 to 14, further comprising adding a cross-linking agent to the mixture to harden the primary and/or secondary shells.

16. The process of any one of claims 9 to 15, further comprising drying the microencapsulated omega-3 polyunsaturated fatty acid glyceride composition by spray-drying or freeze-drying.

17. The process of any one of claims 1 to 16, wherein an anti-oxidant is added to the glycerides.

18. The process of claim 3 or claim 4, wherein the free fatty acids are removed from the partially hydrolysed triglycerides by neutralising the free fatty acids with a base, and extracting the glycerides with organic solvent.

19. A microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, prepared according to the process of any one of claims 1 to 18.

5 20. A microencapsulated omega-3 polyunsaturated fatty acid glyceride composition, the composition comprising:

glycerides comprising at least one of monoglycerides and diglycerides in a total amount of at least 5% based on the total mass of the glycerides, wherein the glycerides comprise at least 30% of omega-3 polyunsaturated fatty acids based on
10 the total mass of glycerides;

an encapsulant which encapsulates droplets of the glycerides, the encapsulant comprising one or more polymer components.

21. The microencapsulated omega-3 polyunsaturated fatty acid glyceride composition
15 of claim 20, wherein the glycerides comprise at least one of monoglycerides and diglycerides in a total amount of at least 10% based on the total mass of the glycerides.

22. The microencapsulated omega-3 polyunsaturated fatty acid glyceride composition
20 of claim 20 or claim 21, wherein the encapsulant is a complex coacervate formed as primary shells encapsulating the droplets, the complex coacervate comprising a protein polymer component and a polyanionic polymer component.

23. The microencapsulated omega-3 polyunsaturated fatty acid glyceride composition
25 of claim 22, wherein agglomerations of the primary shells are encapsulated with secondary shells of the complex coacervate.

24. A nutritional supplement, functional food, medical food or infant formula comprising the microencapsulated omega-3 polyunsaturated fatty acid glyceride composition according to any one of claims 19 to 23.

30 25. Use of a microencapsulated omega-3 polyunsaturated fatty acid glyceride composition according to any one of claims 19 to 23, for the treatment or prevention of a disease or medical condition, wherein the disease or medical

condition is treatable or preventable by ingestion of omega-3 polyunsaturated fatty acids.

Figure 1

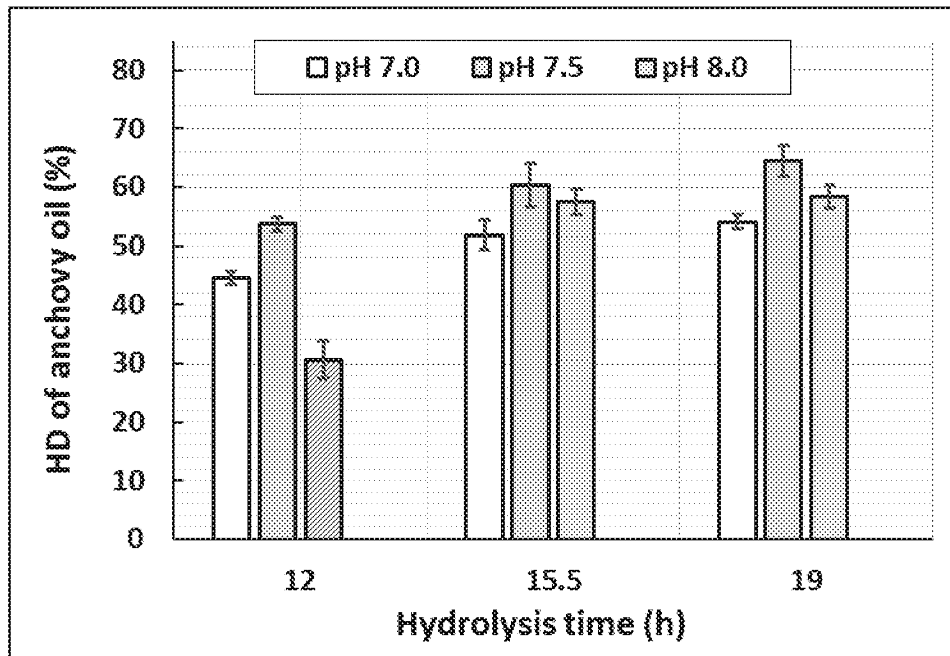


Figure 2

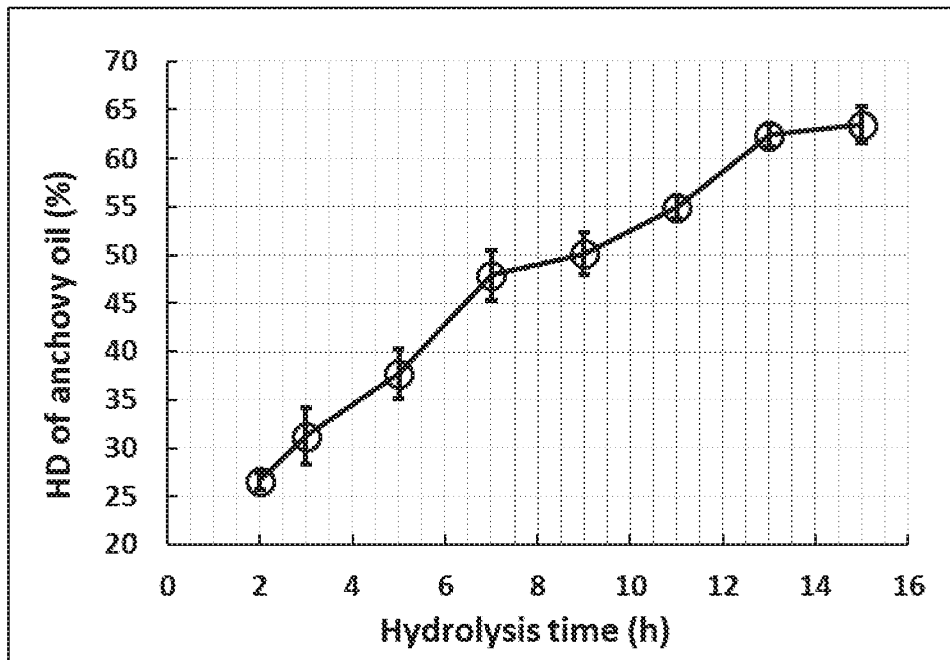


Figure 3

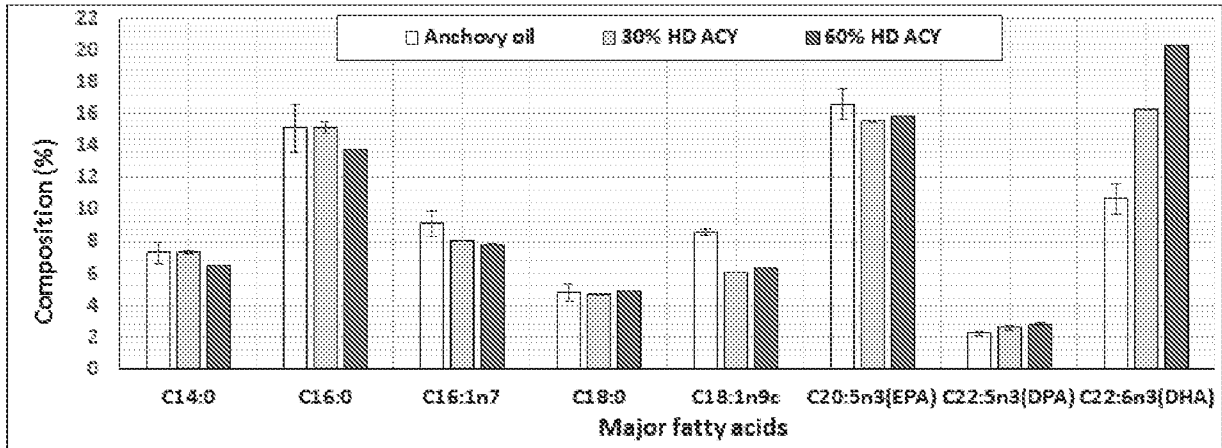


Figure 4

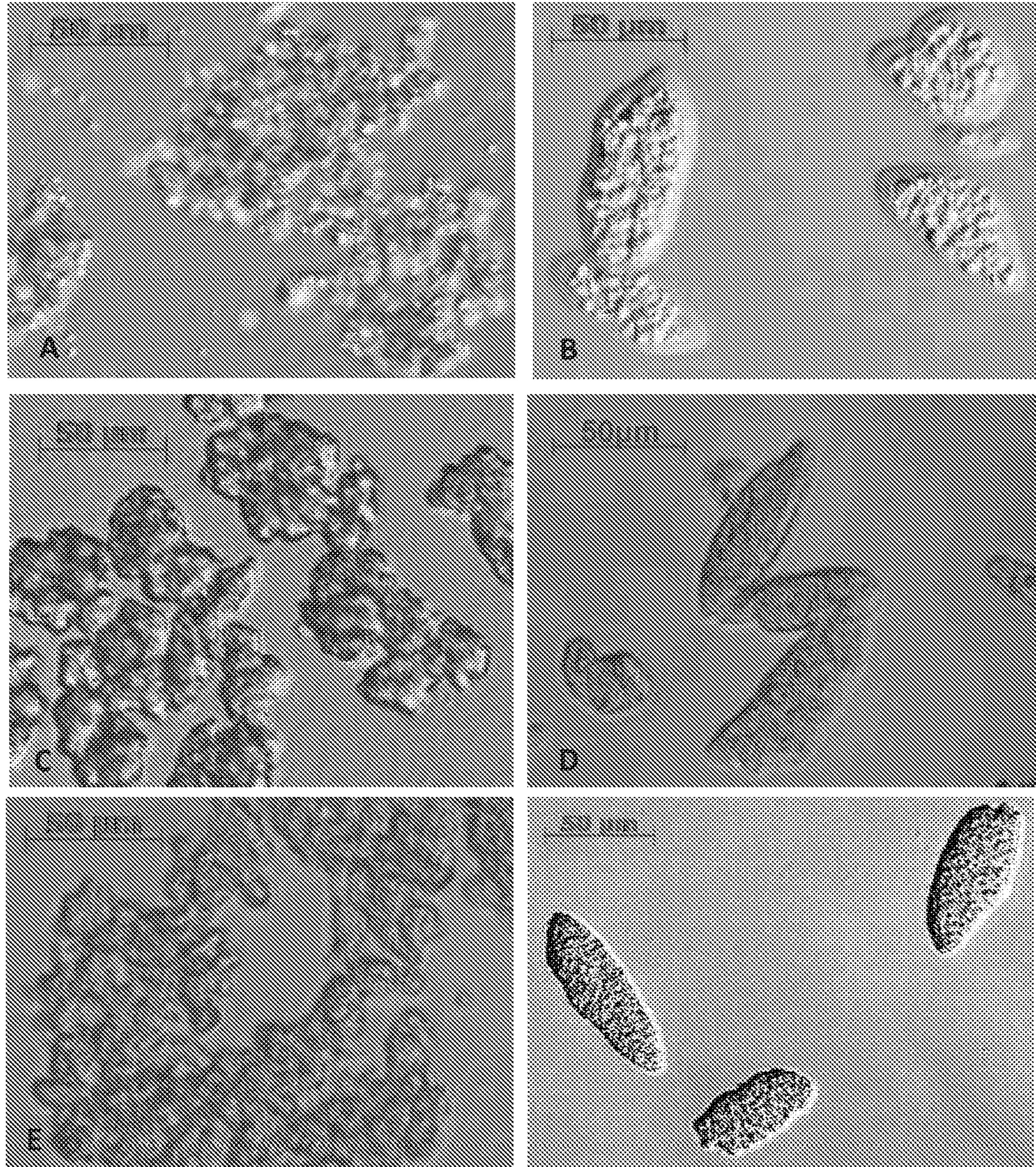
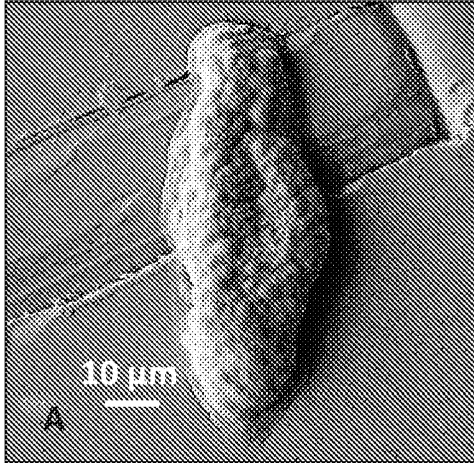
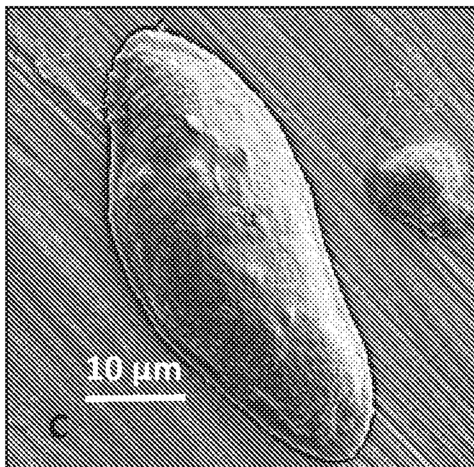


Figure 5





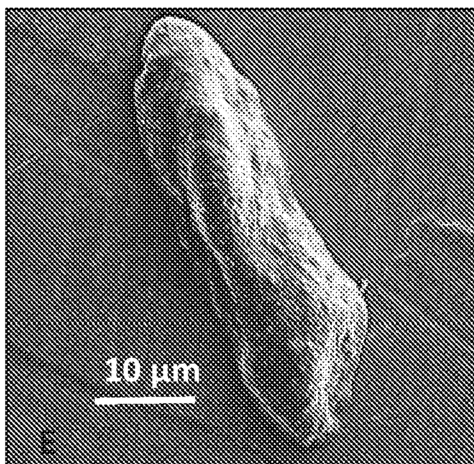
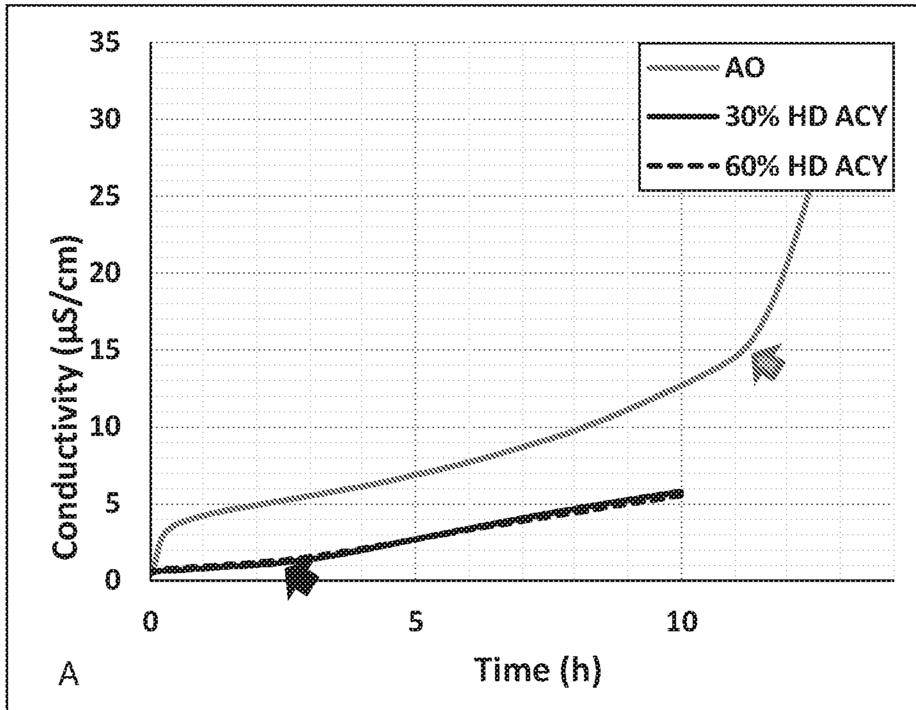


Figure 6



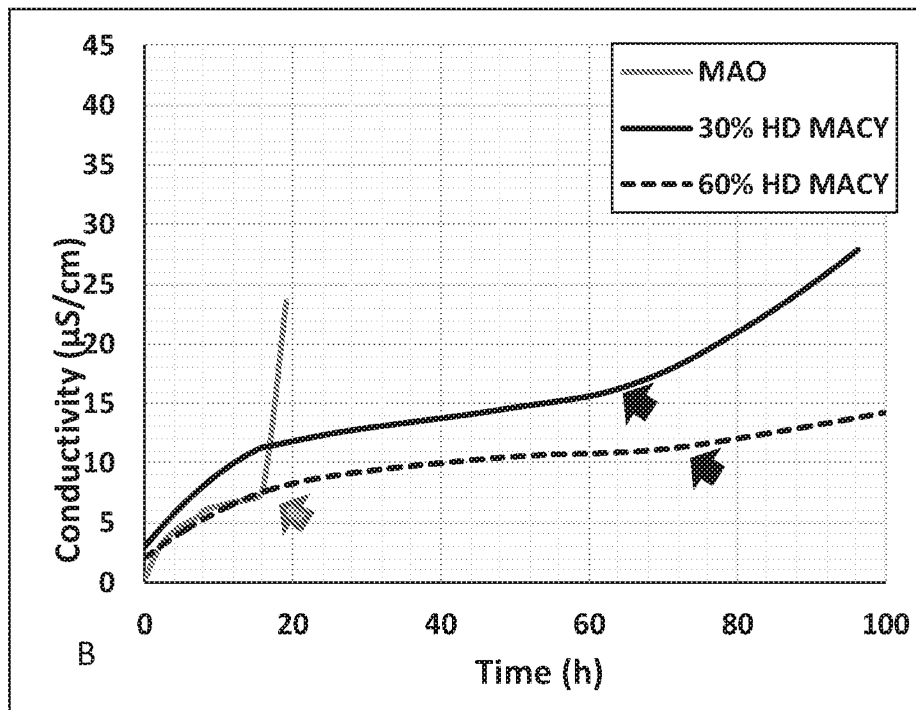


Figure 7

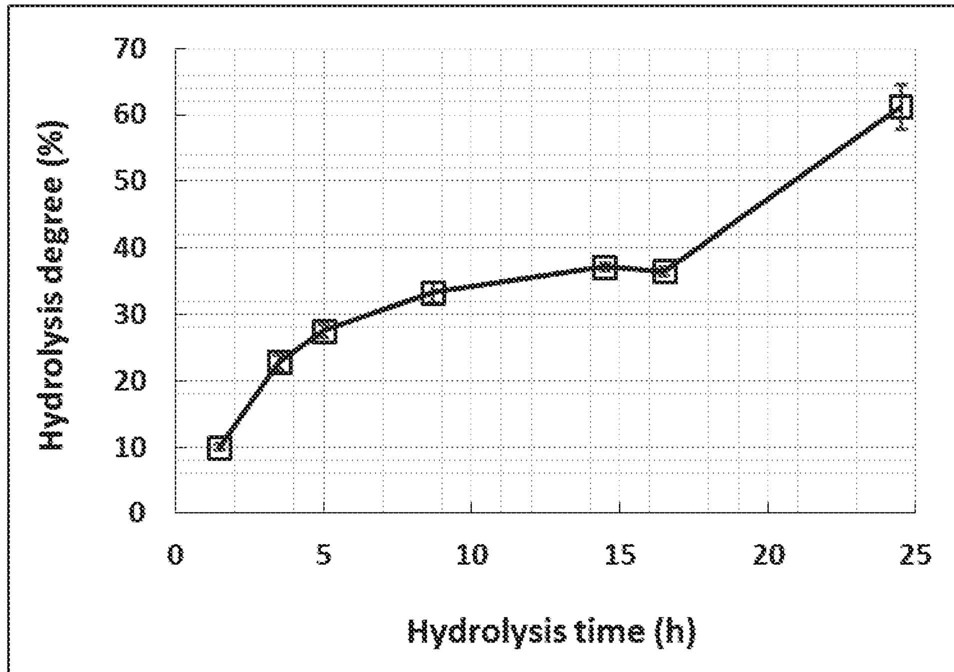


Figure 8

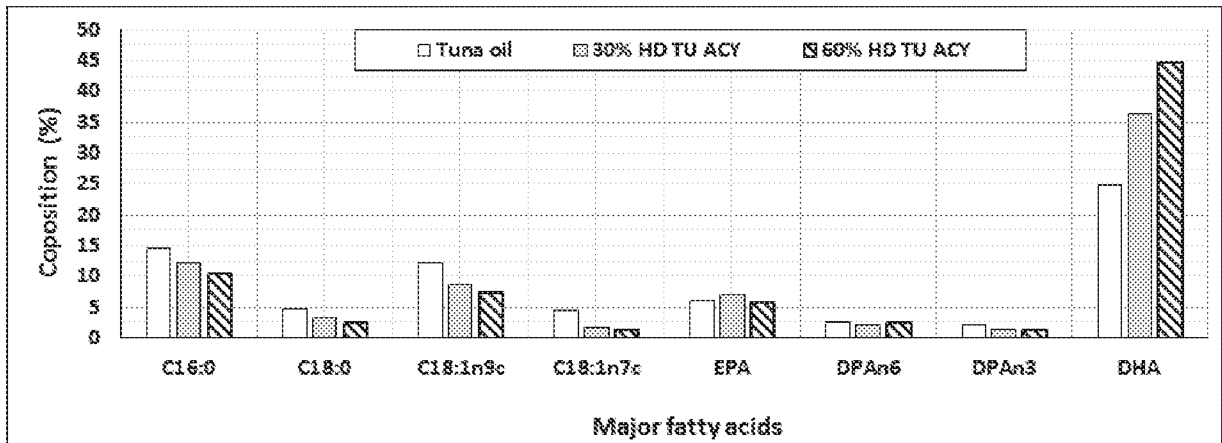


Figure 9

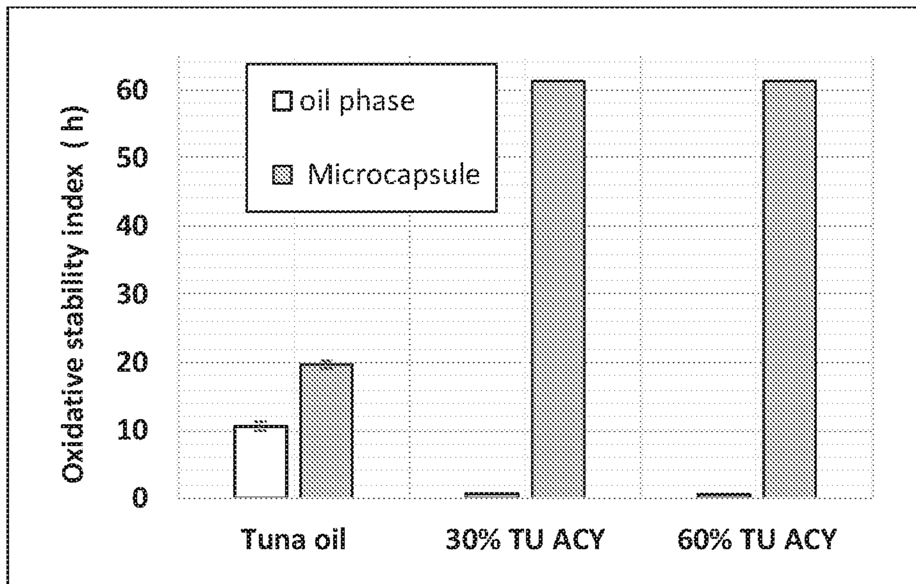
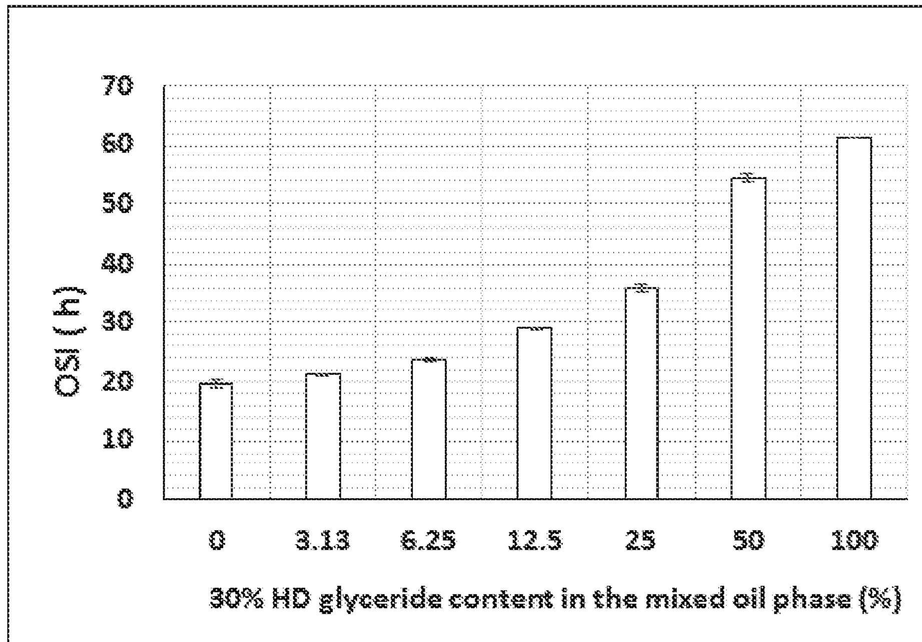


Figure 10



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2017/050454

A. CLASSIFICATION OF SUBJECT MATTER

B01J 13/02 (2006.01) B01J 13/06 (2006.01) A23L 33/12 (2016.01) A61K 9/14 (2006.01) A61K 9/50 (2006.01) A23D 7/06 (2006.01) C11C 1/04 (2006.01) C12N 9/20 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATENW, HCAPLUS, CAPLUS, SCISEARCH, GOOGLE SCHOLAR, ESPACENET (Microencapsulation, Microcapsules, Nanocapsules, Encapsulation, Glycerides, Monoacylglycerol, Diacylglycerol, Triacylglycerol, Monoglyceride, Diglyceride, Triglyceride, PUFA, Polyunsaturated fatty acids, EPA, DHA, Hydrolysis, Lipase, Enzyme, Protein polymer, Polyanionic polymer, Gelatin, Whey protein, Gum arabic, Polyphosphate, Hexametaphosphate, Fish oil, Microbial oil, Algal oil, Tuna oil, Anchovy oil, Edible oil, Nutritional supplement, Infant formula, Food and associated terms)

ESPACENET : Applicant/Inventors Name Search : Deakin Univeristy, Barrow, Wang, Akanbi; Applicant name also searched in internal databases provided by IP Australia.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C   [X] See patent family annex

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Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2017	26 July 2017

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/135501 A1 (CABIO BIOENGINEERING (WUHAN) CO., LTD.) 17 September 2015 See abstract; paras 8-27 and 53-146; Examples; Figures; Claims	1-25
X Y	WO 2011/008097 A1 (FRIESLAND BRANDS B.V.) 20 January 2011 See abstract; page 3, line 22- page 11, line 8; Examples 1-5 and 7-11; claims See as above	1, 2, 9-17 and 19-25 3-8 and 18
X Y	WANG, B, et al., "Optimisation of the Microencapsulation of Tuna Oil in Gelatin–Sodium Hexametaphosphate Using Complex Coacervation", Food Chemistry, 2014, Vol. 158, pages 358-365. See abstract; page 359, column 1, para 2-page 364, column 2, para 1; Table 1; Figures See as above	1, 2, 9-17 and 19-25 3-8 and 18
X Y	WANG, B, et al., "Microencapsulation of Tuna Oil Fortified with the Multiple Lipophilic Ingredients Vitamins A, D_3, E, K_2, Curcumin and Coenzyme Q_{10}", Journal of Functional Foods, 2015, Vol. 19, pages 893-901. See abstract; page 894, column 2, para 2- page 900, column 1, last para; Tables 1-2; Figures See as above	1, 2, 9-17 and 19-25 3-8 and 18
X Y	US 6234464 B1 (KRUMBHOLZ et al) 22 May 2001 See abstract; column 1, line 46-column 2, line 52; Examples 1-5; claims See as above	1, 2, 9-17 and 19-25 3-8 and 18
X Y	WO 2009/029406 A1 (PEPSICO, INC.) 05 March 2009 See abstract, para 15-29; Examples 1-11; claims See as above	1, 2, 9-17 and 19-25 3-8 and 18
X Y	US 2011/0045147 A1 (ALTING et al) 24 February 2011 See abstract; paras 0001; 0007-0096; Examples 1-5; claims See as above	1, 2, 9-17 and 19-25 3-8 and 18
Y	AKANBI, T. O. et al., "Selective Concentration of EPA and DHA Using *Thermomyces Lanuginosus* Lipase is due to Fatty Acid Selectivity and not Regioselectivity", Food Chemistry, 2013, Vol. 138, pages 615-620. See abstract; page 615, column 1, para 1-column 2, para 1; page 616, column 1, para 2-page 619, column 2, para 3; Figures	3-8 and 18
Y	WO 2015/024055 A1 (DEAKIN UNIVERSITY) 26 February 2015 See abstract; page 1, line 13-15; page 1, line 34-page 2, line 26; page 2, line 29-page 10, line 2; page 14, line 24-page 23, line 31; Examples 1-4; claims	3-8 and 18

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2017/050454**

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Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2015/135501 A1	17 September 2015	WO 2015135501 A1	17 Sep 2015
		CN 103882071 A	25 Jun 2014
		CN 103882071 B	05 Oct 2016
		CN 105925627 A	07 Sep 2016
WO 2011/008097 A1	20 January 2011	WO 2011008097 A1	20 Jan 2011
		AU 2010271587 A1	02 Feb 2012
		AU 2010271587 B2	24 Jul 2014
		CN 102480982 A	30 May 2012
		CN 102480982 B	02 Jul 2014
		EP 2453755 A1	23 May 2012
		US 2012128831 A1	24 May 2012
		US 9649611 B2	16 May 2017
US 6234464 B1	22 May 2001	US 6234464 B1	22 May 2001
		DE 19830375 A1	13 Jan 2000
		EP 0970623 A2	12 Jan 2000
		EP 0970623 B1	28 Sep 2005
		NO 992403 A	10 Jan 2000
		NO 327846 B1	05 Oct 2009
WO 2009/029406 A1	05 March 2009	WO 2009029406 A1	05 Mar 2009
		AR 068041 A1	28 Oct 2009
		AU 2008293779 A1	05 Mar 2009
		AU 2008293779 B2	08 Dec 2011
		CN 101790323 A	28 Jul 2010
		CN 105146540 A	16 Dec 2015
		EP 2180795 A1	05 May 2010
		EP 2180795 B1	29 Feb 2012
		EP 2457451 A1	30 May 2012
		HK 1216975 A1	16 Dec 2016
		KR 20100033429 A	29 Mar 2010
		KR 101250827 B1	05 Apr 2013
		US 2009061048 A1	05 Mar 2009

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 9186640 B2	17 Nov 2015
		US 2016206561 A1	21 Jul 2016
US 2011/0045147 A1	24 February 2011	US 2011045147 A1	24 Feb 2011
		AU 2008330337 A1	04 Jun 2009
		CA 2707461 A1	04 Jun 2009
		EP 2214507 A1	11 Aug 2010
		WO 2009070010 A1	04 Jun 2009
WO 2015/024055 A1	26 February 2015	WO 2015024055 A1	26 Feb 2015

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.