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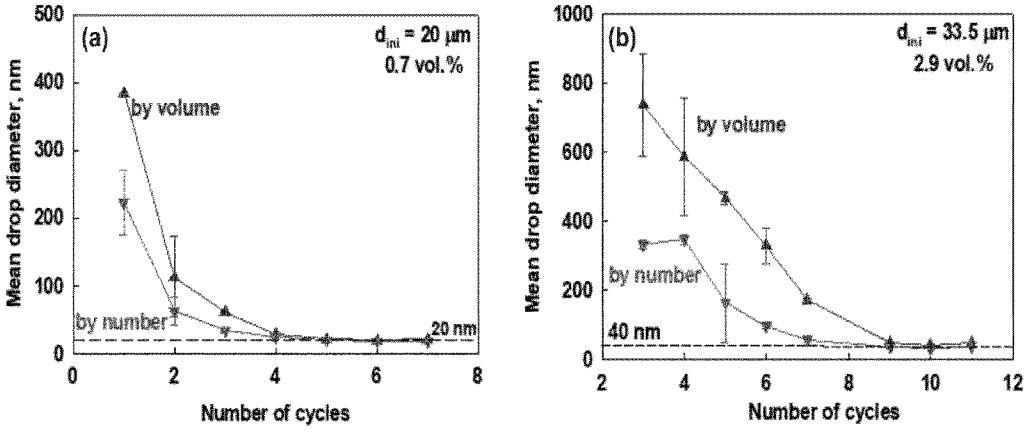
(54) **Title:** OIL-IN-WATER EMULSION OF NANO-SIZED SELF-EMULSIFIED PARTICULATES

FIG 1. Illustrates the drop size decrease observed with bulk emulsions.



(57) **Abstract:** The present invention is directed to an oil-in-water emulsion and methods in which the oil droplets exhibit self-emulsified particulates of a nano-sized diameter and within a narrow size range for used to deliver or store ingredients and provide a new or improved functionality.

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OIL-IN-WATER EMULSION OF NANO-SIZED SELF-EMULSIFIED PARTICULATES

[1] The present invention is directed to an oil-in-water emulsion and methods in which the oil droplets exhibit self-emulsified particulates of a nano-sized diameter and within a narrow size range for used to deliver or store ingredients and provide a new or improved functionality.

INTRODUCTION

[2] Oil-in-water emulsions are widely used in food, cosmetic, agrochemical, zootechnical and pharma industries. Such emulsions can be exploited for many different purposes or applications such as delivering ingredients, or creating a certain feel, texture, fragrance or sensation as well as taste or smoothness. In industry, emulsions also deliver active compounds, such as, flavours, vitamins, antioxidants, nutraceuticals, phytochemicals, drugs, chemicals, enzymes, antibodies, peptibodies, vaccines, viral vectors, hormone therapies, gene-editing techniques, biosimilars and others. Administering active components requires the use of an appropriate vehicle for bringing an effective amount of the active component into a desired place of action. Oil-in-water emulsions are commonly used as delivery systems since they take advantage of the solubility of lipophilic compounds in the oil. Dissolution of active compounds, such as phytosterols, carotenoids or water-insoluble drugs into the oil droplets of emulsions or dispersions not only aids dispersibility (homogeneous incorporation of the active elements), but can also increase their bioavailability. Clinical and animal experiments have demonstrated better efficacy and bioavailability of active compounds, such as drugs and nutrients, when the active compounds are dissolved or solubilised, for instance into nano-emulsions or nano-dispersions.

[3] Recently, particular interest has been expressed in using triglyceride-based emulsions or dispersions such as triglyceride nano-emulsions or nano-dispersions as an alternative to other oils in different industrial applications. Such triglyceride nano-emulsions or nano-dispersions are, however, usually obtained *via* high-pressure homogenization (HPH) or by ultra-sonication (known as high-energy methods) and the typical drop size is in the range of 150 to 300 nm [1-3].

[4] The problem with HPH or sonication methods is the generation of undesirable heat which not only increases the cost of production but also poses real difficulties with commercial scale up. Furthermore, these methods are not applied to temperature-sensitive compounds and the size and range of the drops further restricts applications.

[5] There is a therefore a need for generating further oil-in-water nano-emulsions which are able to address at least some of the above shortcomings.

SUMMARY

[6] Here is disclosed an oil-in-water emulsion, comprising particulates with a diameter range of between about 10 nm to about 500 nm.

[7] In one aspect of the present invention there is provided an oil-in-water emulsion wherein oil droplets of a diameter greater than 1 μm exhibit nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm, due to the presence of:

a. at least one lipophilic surfactant; and/or
b. at least one hydrophilic surfactant.

[8] In some embodiments there is provided an oil-in-water emulsion wherein the oil-in-water emulsion comprises an active ingredient.

[9] In some embodiments there is provided an oil-in-water emulsion wherein the active ingredient is lipophilic.

[10] In some embodiments there is provided an oil-in-water emulsion wherein the active ingredient is between 0.0001% and 50% of the total weight of the emulsion.

[11] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion comprises disseminated oil droplets having nano-sized self-emulsified particulates comprising:

i) oil selected from the group consisting of triglyceride oil, vegetable oil, essential oil, flavouring oil, esters and mixtures thereof;
ii) at least one lipophilic surfactant (Hydrophilic-Lipophilic Balance, HLB < 10) and/or at least one hydrophilic surfactant with HLB value ≥ 10.

[12] In some embodiments there is provided an oil-in-water emulsion wherein the oil is selected from the group consisting of vegetable oils, triglyceride oils, essential oils, flavouring oils, esters, alkanes, paraffins, waxes, diglycerides, phospholipids and mixtures thereof.

[13] In some embodiments there is provided an oil-in-water emulsion wherein the oil is triglyceride oil.

[14] In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride oil is selected from the group consisting of tricaprin ($C_{10}TG$), trilaurin ($C_{12}TG$), trimyristin ($C_{14}TG$), tripalmitin ($C_{16}TG$) and tristearin ($C_{18}TG$) or mixtures thereof.

[15] In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the lipophilic surfactant is between about 0.01% and about 20%, between about 0.01% and about 10%, between about 0.01% and about 5%, between about 0.025% and about 1.9%, between about 0.05% and about 1.8%, between about 0.1% and about 1.5%, between about 0.15% and about 1.25%, between about 0.2% and about 1%, between about 0.4% and about 0.8% of the total weight of the emulsion, and wherein the concentration of the hydrophilic surfactant is between about 0.1% and about 5%, between 0.2% and about 4.5%, between about 0.3% and about 4%, between about 0.4 and about 3.5%, between about 0.5% and about 3%, between about 0.6% and about 2.5%, between about 0.7% and about 2%, between about 0.8% and about 1.5%, between about 0.9% and about 1% of the weight of the total emulsion. In some embodiments there is

provided an oil-in-water emulsion wherein the concentration of the lipophilic surfactant is 0.7%. In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the lipophilic surfactant is 2.9%.

[16] In some embodiments there is provided an oil-in-water emulsion wherein for 1% oil content in the emulsion the preferred concentration of the lipophilic surfactant is between about 0.01% and about 3%, between about 0.025% and about 2.8%, between about 0.05% and about 2.6%, between about 0.1% and about 2.4%, between about 0.2% and about 2%, between about 0.4% and about 2%, between about 0.8% and about 1.8% of the total weight of the emulsion, and wherein the concentration of the hydrophilic surfactant is between about 0.1% and about 5%, between 0.2% and about 4.5%, between about 0.3% and about 4%, between about 0.4 and about 3.5%, between about 0.5% and about 3%, between about 0.6% and about 2.5%, between about 0.7% and about 2%, between about 0.8% and about 1.5%, between about 0.9% and about 1% of the weight of the total emulsion.

[17] In some embodiments there is provided an oil-in-water emulsion wherein for 1% oil content in the emulsion the preferred concentration of the lipophilic surfactant is between about 0.8% and about 1.8% of the total weight of the emulsion.

[18] In some embodiments there is provided an oil-in-water emulsion wherein for 1% oil content in the emulsion the preferred concentration of lipophilic surfactant is about 1.5% of the weight of the total emulsion and the concentration of the hydrophilic surfactant is about 1% of the weight of the total emulsion.

[19] In some embodiments, for different oil content (volume fraction ϕ), the preferred amount of surfactant *c* for the hydrophilic surfactant could be estimated by the formula [4]: $c = 6\varphi\Gamma/[(1-\varphi)\, d_{32}]$ while for the lipophilic surfactant $c = 6\Gamma/d_{32}$

[20] , where c is the minimal needed surfactant concentration; φ is the oil volume fraction; Γ is the monolayer surfactant adsorption and d_{32} is the mean volume-surface diameter of the droplets in the produced emulsion [4].

[21] In at least one embodiment, the concentration of the lipophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[22] In at least one embodiment, the concentration of the hydrophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[23] In some embodiments there is provided an oil-in-water emulsion wherein the lipophilic surfactant is at least one lipophilic surfactant selected from the group consisting of sorbitan monoalkylate (C_nSorb) where *n* is between 12 and 18; polyoxyethylene glycol hexadecyl ether ($C_{16}EO_2$), polyoxyethylene glycol stearyl ether ($C_{18}EO_2$), polyethylene glycol monooleyl ($C_{18:1}EO_2$), oleyl phosphate, oleyl acetate and 1-oleoyl-*rac*-glycerol (monoolein), glucoside-based

surfactants, diglyceride surfactants, phospholipid surfactants, as well as surfactants that are not only used for stabilization only but where the surfactant is also an active ingredient itself.

[24] In some embodiments there is provided an oil-in-water emulsion wherein the hydrophilic surfactant is at least one hydrophilic surfactant selected from the group consisting of: nonionic polyoxyethylene alkyl ether (C_nEO_m), polyoxyethylene sorbitan monoalkylate ($C_nSorbEO_m$) where *n* is between 12 and 40 and where *m* is between 20 and 50; polyethylene glycol monooleyl ether ($C_{18:1}EO_m$) where *m* is between 7 and 20; polyoxyethylene octyl phenyl ether; anionic sodium dodecyl sulfate (SDS); sodium lauryl ether sulfate (SLES); cationic surfactant cetyltrimethyl ammonium bromide (CTAB); and cocamidopropyl betaine (CAPB), glucoside-based surfactants (such as alkyl polyglycosides, glycopeptides and esters), peptide amphiphiles, aminoacid surfactants, diglyceride surfactants, phospholipid surfactants, as well as surfactants that are not only used for stabilization only but where the surfactant is also an active ingredient itself, and surfactants which contain reactive groups and thus could be used subsequently for crosslinking a surface layer around an emulsified droplet.

[25] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion is exposed to at least two cooling-heating cycles, at least three cooling-heating cycles, at least four cooling-heating cycles, at least five cooling-heating cycles, at least six cooling-heating cycles, at least seven cooling-heating cycles, at least eight cooling-heating cycles or more cooling-heating cycles.

[26] In some embodiments of the method of the present invention, the heating temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of the oil.

[27] In some embodiments of the method of the present invention, the cooling temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of the oil.

[28] In some embodiments of the method of the present invention, the heating temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of the triglyceride.

[29] In some embodiments of the method of the present invention, the cooling temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of the triglyceride.

[30] In some embodiments there is provided an oil-in-water emulsion wherein the nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm are stabilised with at least one lipophilic surfactant and at least one hydrophilic surfactant. In some embodiment, there is provided an emulsion comprising stabilised nano-sized self-emulsified particulates have a diameter of between about 12 nm to about 480 nm, about 14 nm to about 460

nm, about 16 nm to about 440 nm, about 18 nm to about 420 nm, about 20 nm to about 400 nm, about 22 nm to about 380 nm, about 24 nm to about 360 nm, about 26 nm to about 340 nm, about 28 nm to about 320 nm, 30 nm to about 300nm, about 35 nm to about 280 nm, about 40 nm of about 260nm, about 45 nm to about 240 nm, about 50 nm to about 220, about 55 nm to about 200, about 60 nm to about 180 nm, about 65 nm to about 160 nm, about 70 nm to about 140 nm, about 75 nm to about 120 nm, about 80 nm to about 100 nm.

[31] In some embodiments there is provided an oil-in-water emulsion wherein over 70% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 75% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 80% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 85% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 90% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 95% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 96% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 97% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 98% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 99% of the nano-sized self-emulsified particulates have a diameter in the range of about 10 nm to about 100 nm.

[32] In some embodiments, there is provided an oil-in-water emulsion wherein the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm to about 50 nm.

[33] In some embodiments there is provided an oil-in-water emulsion wherein over 70% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 75% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 80% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 85% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 90% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein

over 95% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 96% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 97% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 98% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 99% of the nano-sized self-emulsified particulates have a diameter of about 20 nm to about 50 nm. In some embodiments, there is provided an oil-in-water emulsion wherein the nano-sized self-emulsified particulates exhibit a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 70% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 75% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 80% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 85% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 90% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 95% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 96% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 97% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 98% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm. In some embodiments there is provided an oil-in-water emulsion wherein over 99% of the nano-sized self-emulsified particulates have a diameter of about 10 nm to about 40 nm.

[34] In some embodiments, there is provided an oil-in-water emulsion wherein the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm.

[35] In some embodiments, there is provided an oil-in-water emulsion wherein over 70% or more of the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm. In some embodiments, there is provided an oil-in-water emulsion wherein over 75% or more of the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm. In some embodiments, there is provided an oil-in-water emulsion wherein over 80% or more of the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm. In some embodiments, there is provided an oil-in-water emulsion wherein over 85% or more of the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm. In some embodiments, there is provided an oil-in-water emulsion wherein over 90% or more of the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm.

In some embodiments, there is provided an oil-in-water emulsion wherein over 95% or more of the nano-sized self-emulsified particulates exhibit a diameter of about 20 nm.

[36] In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the stabilising lipophilic surfactant is between about 0.01% and about 20%, between about 0.01% and about 10%, between about 0.01% and about 5%, between about 0.01% and about 2%, between about 0.025% and about 1.9%, between about 0.05% and about 1.8%, between about 0.1% and about 1.5%, between about 0.15% and about 1.25%, between about 0.2% and about 1%, between about 0.4% and about 0.8% of the total weight of the emulsion, and wherein the concentration of the stabilising hydrophilic surfactant is between about 0.1% and about 5%, between 0.2% and about 4.5%, between about 0.3% and about 4%, between about 0.4 and about 3.5%, between about 0.5% and about 3%, between about 0.6% and about 2.5%, between about 0.7% and about 2%, between about 0.8% and about 1.5%, between about 0.9% and about 1% of the weight of the total emulsion.

[37] In at least one embodiment, the concentration of the stabilising lipophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[38] In at least one embodiment, the concentration of the stabilising hydrophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[39] In some embodiments there is provided an oil-in-water emulsion wherein the cooling rate of the cooling-heating cycle is between about 0.01 K min^{-1} and about 1000 K min^{-1}, between about 0.025 K min^{-1} and about 50 K min^{-1}, between about 0.05 K min^{-1} and about 40 K min^{-1}, between about 0.075 K min^{-1} and about 35 K min^{-1}, between about 0.1 K min^{-1} and about 30 K min^{-1}, between about 0.125 K min^{-1} and about 25 K min^{-1}, between about 0.2 K min^{-1} and about 20 K min^{-1}, between about 0.4 K min^{-1} and about 15 K min^{-1}, between about 0.5 K min^{-1} and about 10 K min^{-1}, between about 0.75 K min^{-1} and about 7.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 2.5 K min^{-1}, between about 1.5 K min^{-1} and about 2 K min^{-1}. In some embodiments the cooling rate is about 1 K min^{-1}.

[40] In some embodiments there is provided an oil-in-water emulsion wherein the self-emulsification can happen in the frozen state without heating, or where the heating rate of the cooling-heating cycle is between about 0.01 K min^{-1} and about 1000 K min^{-1}.

[41] In some embodiments there is provided an oil-in-water emulsion wherein self-emulsification occurs at constant temperature.

[42] In some embodiments there is provided an oil-in-water emulsion wherein self-emulsification occurs at between about 33°C and about 37°C.

[43] In some embodiments there is provided an oil-in-water emulsion wherein self-emulsification occurs at about 35°C.

[44] In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride is at least one selected from the group consisting of trimyristine, trilaurine, tristearine and tripalmitine.

[45] In some embodiments there is provided an oil-in-water emulsion wherein the active ingredient is at least one selected from the group consisting of flavours, flavour precursors, aromas, aroma precursors, taste enhancers, salts, sugars, amino-acids, polysaccharides, enzymes, peptides, proteins or carbohydrates, food supplements, food additives, hormones, bacteria, plant extracts, medicaments, drugs, pharmaceuticals, bio-pharmaceutical, biosimilars, vaccines, antigens, antibodies (Abs), peptibodies, antibody-drug-conjugates (ADCs), nutrients, chemicals for agro-chemical or cosmetic applications, carotenoids, vitamins, antioxidants or nutraceuticals selected from the group comprising of lutein, lutein esters, [beta]-carotene, tocopherol, tocopherol acetate, tocotrienol, lycopene, Co-Q10, flax seed oil, fish oil, omega-3 oils, omega-6 oils, DHA, EPA, arachidonic-rich oils, LCPUFA oils, menthol, mint oil, lipoic acid, vitamins, polyphenols and their glycosides, ester and/or sulphate conjugates, isoflavones, flavonols, flavanones and their glycosides such as hesperidin, flavan 3-ols comprising catechin monomers and their gallate esters such as epigallocatechin gallate and their procyanidin oligomers, vitamin C, vitamin C palmitate, vitamin A, vitamin B12, vitamin D, CC-and [gamma]-polyunsaturated fatty acids, phytosterols, esterified phytosterol, non-esterified phytosterol, zeaxanthine, caffeine and a combination thereof.

[46] The present emulsions are suitable for use in the food industry such as ice-cream sector, mayonnaise sector or margarine sector.

[47] In some embodiments there is provided an oil-in-water emulsion for used in the food industry.

[48] In some embodiments there is provided an oil-in-water emulsion for use in making an ice-cream product.

[49] In some embodiments there is provided an oil-in-water emulsion for use in making mayonnaise.

[50] In some embodiments there is provided an oil-in-water emulsion for use in making margarine.

[51] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion is dried in a powder form.

[52] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion is a starting material, an intermediate product or an additive to a final product.

[53] In some embodiments there is provided an oil-in-water emulsion wherein the starting material, the intermediate product or additive to a final product is a pharmaceutical, agro-chemical, consumer, food supplement or cosmetic starting material, the intermediate product or additive to a final product.

[54] In some embodiments there is provided an oil-in-water emulsion wherein the starting material, the intermediate product or additive to a final product has an increased shelf-life.

[55] In some embodiments there is provided an oil-in-water emulsion for use in enhancing stability, protection against chemical or enzymatic degradation or oxidation of an active ingredient in the oil-in-water emulsion.

[56] In some embodiments there is provided an oil-in-water emulsion for use in enhancing bioavailability, bioaccessibility or absorption of active ingredient during digestion.

[57] In some embodiments there is provided an oil-in-water emulsion for use in controlled release, burst release, or sustained release of an active ingredient during consumption or digestion.

[58] In some embodiments there is provided an oil-in-water emulsion for use in controlled release of aroma or flavour, burst release of aroma or flavour, or sustained release of aroma or flavour to create new or improved sensory or taste properties.

[59] In some embodiments there is provided an oil-in-water emulsion for use in creating different taste, different texture, mouth feel, mouth coating, or creaminess sensation.

[60] In some embodiments there is provided an oil-in-water emulsion for use in taste masking, off taste masking, flavour masking, off flavour masking, taste modulation or flavour modulation.

[61] Here we also disclose a one aspect a pharmaceutical combination comprising the oil-in-water emulsion of the present invention and at least one further pharmaceutically acceptable surfactant, excipient or additive.

[62] In some embodiments there is provided a pharmaceutical combination for use in medicine.

[63] Here we also describe a low-energy method which leads to oil-in-water nano-emulsions, containing particulates with a diameter range between about 10 nm to about 500 nm. The method requires the usage of hydrophilic and/or lipophilic surfactant(s) and controlled variation of the emulsion temperature around melting temperature of the oil. In some embodiments of the method of the present invention, the melting temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of the oil. In some embodiments of the method of the present invention, the freezing temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of the oil.

[64] According to one aspect of the present invention, there is provided a method for producing an oil-in-water emulsion according to the present invention, wherein the method comprising the steps of:

a. preparing an initial emulsion of a lipophilic phase in a hydrophilic phase to form oil droplets of a diameter greater than 1 μm in the presence of at least one lipophilic surfactant and/or at least one hydrophilic surfactant; and
b. treating the initial emulsion to form an oil-in-water emulsion comprising nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm.

[65] In some embodiments of the method of the present invention, wherein the initial emulsion comprises an intermediate metastable phase.

[66] In some embodiments of the method of the present invention, the initial emulsion is subjected to at least one fast cooling cycle.

[67] In some embodiments of the method of the present invention, the oil is selected from the group consisting of vegetable oils, triglyceride oils, essential oils, flavouring oils, esters, alkanes, paraffins, waxes, diglycerides, phospholipids and mixtures thereof.

[68] In some embodiments of the method of the present invention, the oil is triglyceride oil.

[69] In some embodiments of the present invention, the method requires a combination of hydrophilic surfactants and lipophilic surfactants and controlled variation of the emulsion temperature around the cooling-heating or melting-freezing temperature of the triglyceride.

[70] In some embodiments of the method of the present invention, the initial emulsion is treated by at least two cooling-heating cycles, at least three cooling-heating cycles, at least four cooling-heating cycles, at least five cooling-heating cycles, at least six cooling-heating cycles, at least seven cooling-heating cycles, at least eight cooling-heating cycles or more cooling-heating cycles.

[71] In some embodiments of the method of the present invention, the heating temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of the triglyceride.

[72] In some embodiments of the method of the present invention, the cooling temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of the triglyceride.

[73] In some embodiments of the method of the present invention, the nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm are stabilised. In some embodiments of the method of the present invention, the stabilised nano-sized self-emulsified particulates have a diameter of between about 12 nm to about 480 nm, about 14 nm to about 460 nm, about 16 nm to about 440 nm, about 18 nm to about 420 nm, about 20 nm to about 400 nm, about 22 nm to about 380 nm, about 24 nm to about 360 nm, about 26 nm to about 340 nm, about 28 nm to about 320 nm, 30 nm to about 300nm, about 35 nm to about 280 nm, about 40

nm of about 260nm, about 45 nm to about 240 nm, about 50 nm to about 220, about 55 nm to about 200, about 60 nm to about 180 nm, about 65 nm to about 160 nm, about 70 nm to about 140 nm, about 75 nm to about 120 nm, about 80 nm to about 100 nm.

[74] In some embodiments of the method of the present invention, the cooling rate is between about 0.01 K min^{-1} and about 1000 K min^{-1}, between about 0.01 K min^{-1} and about 50 K min^{-1}, between about 0.025 K min^{-1} and about 45 K min^{-1}, between about 0.05 K min^{-1} and about 40 K min^{-1}, between about 0.075 K min^{-1} and about 35 K min^{-1}, between about 0.1 K min^{-1} and about 30 K min^{-1}, between about 0.125 K min^{-1} and about 25 K min^{-1}, between about 0.2 K min^{-1} and about 20 K min^{-1}, between about 0.4 K min^{-1} and about 15 K min^{-1}, between about 0.5 K min^{-1} and about 10 K min^{-1}, between about 0.75 K min^{-1} and about 7.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 2.5 K min^{-1}, between about 1.5 K min^{-1} and about 2 K min^{-1}. In some embodiments the cooling rate is about 1 K min^{-1}.

[75] In some embodiments of the method of the present invention, the heating rate is between about 0.01 K min^{-1} and about 10 K min^{-1}, between about 0.025 K min^{-1} and about 9.5 K min^{-1}, between about 0.05 K min^{-1} and about 9 K min^{-1}, between about 0.075 K min^{-1} and about 8.5 K min^{-1}, between about 0.1 K min^{-1} and about 8 K min^{-1}, between about 0.125 K min^{-1} and about 7.5 K min^{-1}, between about 0.2 K min^{-1} and about 7 K min^{-1}, between about 0.4 K min^{-1} and about 6.5 K min^{-1}, between about 0.5 K min^{-1} and about 6 K min^{-1}, between about 0.75 K min^{-1} and about 5.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 4.5 K min^{-1}, between about 1.5 K min^{-1} and about 4 K min^{-1}, between about 2 K min^{-1} and about 3 K min^{-1}. In some embodiments, the heating rate of the cooling-heating cycle is about 1 K min^{-1}.

BRIEF DESCRIPTION OF THE DRAWINGS

[76] **FIG 1** Illustrates the drop or droplet size decrease observed with large scale or bulk emulsions - FIG 1(a) and FIG 1(b).

[77] **FIG 2** Depicts the particle disintegration observed upon storage of the emulsion.

DETAILED DESCRIPTION

[78] Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this disclosure pertains.

[79] As used herein, certain terms may have the following defined meanings.

[80] As used in the specification and claims, the singular form “a,” “an” and “the” include singular and plural references unless the context clearly dictates otherwise. For example, the term “a surfactant” or “a triglyceride” includes a single surfactant or triglyceride as well as a plurality of surfactants or triglycerides, including mixtures thereof.

[81] As used herein, the term "comprising" means including, made up of, composed of encompass, consist of, constitute and incorporate.

[82] All numbers or numerals as used herein that indicate amounts, ratios of materials, physical properties of materials, and/or use are to be understood as modified or qualified by the term "about," except as otherwise explicitly indicated.

[83] As used herein, the term "about" includes the recited number or number and +/- 10% from the recited numeral or number. By way of non-limiting example, the term "about ten (10)" would encompass nine (9) to eleven (11) or 9-11.

[84] As used in the specification and claims, the term “self-emulsification” refers to a reduction of size or diameter of oil droplets or drop as a result of temperature changes without physical or mechanically induced agitation or disruption of the drops. In some embodiments self-emulsification can be attained by cooling and/or heating the drops. For instance, self-emulsification of oil droplets of a diameter greater than 1 μm can undergo a reduction in the size or diameter, in the range of between about 10 nm to about 500 nm without physically or mechanically induced agitation or disruption of the droplets. In some embodiments, the temperature could be set stationary (e.g. with cooling the droplets at a substantially fixed or constant temperature – Example 2). For instance, self-emulsification can occur without heating and/or cooling cycle. In some embodiments there is provided an oil-in-water emulsion wherein self-emulsification occurs at constant temperature. In some embodiments there is provided an oil-in-water emulsion wherein self-emulsification occurs at between about 33°C and about 37°C. In some embodiments there is provided an oil-in-water emulsion wherein self-emulsification occurs at about 35°C. As used herein the terms “drop” or “droplet” are used interchangeably unless stated otherwise or the context dictates otherwise.

[85] As used herein “size” is measured on a volumetric-averaged basis, unless otherwise specified. The “particulate” or “droplet” in the present invention is a concept, unless otherwise specified, which can encompass not only a particulate or droplet but a particulate or droplet emulsion composed or comprising of agglomeration of such particulates or droplets.

[86] In some embodiments, the size is measured using any method know in the art which is suitable for the particular purpose. In some embodiments, the size of the droplets, for example in the case of micro-size dimensions, is determined using optical microscopy [5]. In some embodiments the size of the particulates, for example in the case of nano-size dimentions, is determined using dynamic light scattering (DLS) by statistically corelating the Brownian motion fluctuations of the particulates [6].

[87] As used in the specification and claims, the term “exhibit” refers to self-emulsified emulsion which displays or incorporates nano-sized particulates of diameter in the range of about 10 nm to about 500 nm.

[88] In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 1000 Celsius degrees per minute, or higher. In some embodiments, the

temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 2000 Celsius degrees per minute (^{0}C/min), or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 3000 ^{0}C/min, or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 4000 ^{0}C/min, or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 5000 ^{0}C/min, or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 6000^{0}C/min, or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 8000 ^{0}C/min, or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 9000 ^{0}C/min, or higher. In some embodiments, the temperature can be changed at a controlled cooling rate, e.g. between 0.0001 and 10000 ^{0}C/min.

[89] As used in the specification and claims, the term "lipophilic surfactant" refers to surfactants which are soluble in lipids or oils. For instance, lipophilic surfactants include sorbitan monoalkylates (C_nSorb) with *n* varied between 12 and 18, polyoxyethylene glycol hexadecyl ether ($C_{16}EO_2$), polyoxyethylene glycol stearyl ether ($C_{18}EO_2$), polyethylene glycol monooleyl ether with 2 ethoxy units ($C_{18:1}EO_2$), oleyl phosphate, oleyl acetate and 1-oleoyl-*rac*-glycerol (monoolein). Without wishing to be bound by theory, surfactants with HLB<10 are oil soluble or lipophilic (see Table 2 and Examples).

[90] As used in the specification and claims, the term "hydrophilic surfactant" refers to water-soluble nonionic, anionic, cationic or zwitterionic surfactants. For instance, nonionic hydrophilic surfactants includes, polyoxyethylene alkyl ethers (C_nEO_m) and polyoxyethylene sorbitan monoalkylates (C_nSorbEO_m) where *n* can be any one of between 12 and 40, and where *m* can be any one of between 20 and 50, polyethylene glycol monooleyl ethers ($C_{18:1}EO_m$) where *m* can be any one of between 7 and 20, and polyoxyethylene octyl phenyl ether (trade name Triton X100); anionic hydrophilic surfactants include sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES); cationic hydrophilic surfactants include cetyltrimethyl ammonium bromide (CTAB); and zwitterionic hydrophilic surfactants include cocamidopropyl betaine (CAPB). Without wishing to be bound by theory, surfactants with HLB $\geq$ 10 are water soluble or hydrophilic (see Table 2 and Examples).

[91] In some embodiments, the at least one lipophilic surfactant and the at least one hydrophilic surfactant, have a resulting HLB value (Hydrophilic-Lipophilic Balance) of less (<) than any one of 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 or less.

[92] In some embodiments the at least one lipophilic surfactant HLB value (Hydrophilic-Lipophilic Balance) < 10, and the at least one hydrophilic surfactant HLB value is (greater or equal to) $\geq$ 10.

[93] As used in the specification and claims, the term "active ingredient" refers to at least one ingredient selected from but are not limited to, lipophilic active compounds or a salt, isomer, ester, ether or other derivative thereof selected from: a) acetylcholinesterase inhibitors; b) analgesics and nonsteroidal antiinflammatory agents (NSAIA) selected from aloxiprin, auranofin,

azapropazone, benorylate, capsaicin, celecoxib, diclofenac, diflunisal, etodolac, fenbufen, fenoprofen calcium, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, leflunomide, meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxaprozin, oxyphenbutazone, phenylbutazone, piroxicam, rofecoxib, sulindac, tetrahydrocannabinol, tramadol and tromethamine; c) anthelminthics selected from albendazole, bephenium hydroxynaphthoate, cambendazole, dichlorophen, fenbendazole, ivermectin, mebendazole, oxamniquine, oxfendazole, oxantel embonate, praziquantel, pyrantel embonate and thiabendazole; d) antiacne agents such as isotretinoin and tretinoin; e) anti-anginal agents selected from amyl nitrate, glyceryl trinitrate (nitroglycerin), isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate, and ubidecarenone (coenzyme Q10); f) anti-asthma agents selected from zileuton, zafirlukast, terbutaline sulfate, montelukast, and albuterol; g) antibacterial agents, including antibiotics, selected from alatrofloxacin, azithromycin, aztreonum, baclofen, benzathine penicillin, cefixime, cefuraxime axetil, cinoxacin, ciprofloxacin HCl, clarithromycin, clofazimine, cloxacillin, demeclocycline, dirithromycin, doxycycline, erythromycin, ethionamide, furazolidone, grepafloxacin, imipenem, levofloxacin, lorefloxacin, moxifloxacin HCl, nalidixic acid, nitrofurantoin, norfloxacin, ofloxacin, phenoxymethyl penicillin, rifabutine, rifampicin, rifapentine, sparfloxacin, spiramycin, sulphabenzamide, sulphadoxine, sulphamerazine, sulphacetamide, sulphadiazine, sulphafurazole, sulpha-methoxazole, sulphapyridine, tetracycline, trimethoprim, trovafloxacin, and vancomycin; h) anticancer agents and immunosuppressants selected from abarelix, aldesleukin, alemtuzumab, alitretinoin, all-trans retinoic acid (ATRA), altretamine, amifostine, aminoglutethimide, amsacrine, anastrozole, arsenic trioxide, asparaginase, azacitidine, azathioprine, BCG Live, bevacuzimab (avastin), bexarotene, bicalutamide, bisantrene, bleomycin, bortezomib, busulfan, calusterone, camptothecin, capecitabine, carboplatin, carmustine, celecoxib, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cyclosporin, cytarabine, dacarbazine, dactinomycin, darbepoetin alfa, daunorubicin, denileukin, dexrazoxane, docetaxel, doxorubicin (neutral), doxorubicin HCl, dromostanolone propionate, ellipticine, enlimomab, estramustine, epirubicin, epoetin alfa, erlotinib, estramustine, etoposide, exemestane, filgrastim, floxuridine fludarabine, fulvestrant, gefitinib, gemcitabine, gemtuzumab, goserelin acetate, histrelin acetate, hydroxyurea, ibritumomab, idarubicin, ifosfamide, imatinib mesylate, interferon alfa-2a, interferon alfa-2b, irinotecan, lenalidomide, letrozole, leucovorin, leuprolide acetate, levamisole, lomustine, megestrol acetate, melphalan, mercaptopurine, mesna, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, mofetil mycophenolate, nandrolone, nelarabine, nilutamide, nofetumomab, oprelvekin, oxaliplatin, paclitaxel, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, pentostatin, pipobroman, plicamycin, porfimer sodium, procarbazine, quinacrine, rasburicase, rituximab, sargramostim, sirolimus, sorafenib, streptozocin, sunitinib maleate, tacrolimus, tamoxifen citrate, temozolomide, teniposide, testolactone, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, zoledronate, and zoledronic acid; i) anticoagulants selected from cilostazol, clopidogrel, dicumarol, dipyridamole, nicoumalone, oprelvekin, phenindione, ticlopidine, and tirofiban; j) antidiabetics selected from acetohexamide,

chlorpropamide, glibenclamide, gliclazide, glipizide, glimepiride, glyburide, miglitol, pioglitazone, repaglinide, rosiglitazone, tolazamide, tolbutamide and troglitazone; k) antifungal agents selected from amphotericin, butenafine HCl, butoconazole nitrate, clotrimazole, econazole nitrate, fluconazole, flucytosine, griseofulvin, itraconazole, ketoconazole, miconazole, natamycin, nystatin, sulconazole nitrate, oxiconazole, terbinafine HCl, terconazole, tioconazole and undecenoic acid; l) antihypertensive agents selected from amlodipine, benidipine, benezepril, candesartan, captopril, darodipine, dilitazem HCl, diazoxide, doxazosin HCl, enalapril, eposartan, losartan mesylate, felodipine, fenoldopam, fosenopril, guanabenz acetate, irbesartan, isradipine, lisinopril, minoxidil, nicardipine HCl, nifedipine, nimodipine, nisoldipine, phenoxybenzamine HCl, prazosin HCl, quinapril, reserpine, terazosin HCl, telmisartan, and valsartan; m) antithyroid agents selected from carbimazole and propylthiouracil; n) antiviral agents selected from abacavir, amprenavir, delavirdine, efavirenz, indinavir, lamivudine, nelfinavir, nevirapine, ritonavir, saquinavir, and stavudine; o) anxiolytics, sedatives, hypnotics and neuroleptics selected from alprazolam, amylobarbitone, barbitone, bentazepam, bromazepam, bromperidol, brotizolam, butobarbitone, carbromal, chlordiazepoxide, chlormethiazole, chlorpromazine, chlorprothixene, clonazepam, clobazam, clotiazepam, clozapine, diazepam, droperidol, ethinamate, flunanisone, flunitrazepam, triflupromazine, flupenthixol decanoate, fluphenthixol decanoate, flurazepam, gabapentin, haloperidol, lorazepam, lormetazepam, medazepam, meprobamate, mesoridazine, methaqualone, methylphenidate, midazolam, molindone, nitrazepam, olanzapine, oxazepam, pentobarbitone, perphenazine pimozide, prochlorperazine, propofol, pseudoephedrine, quetiapine, risperidone, sertindole, sulpiride, temazepam, thioridazine, triazolam, zolpidem, and zopiclone; p) beta-blockers selected from acebutolol, alprenolol, atenolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol and propranolol; q) cardiac inotropic agents selected from anrinone, digitoxin, digoxin, enoximone, lanatoside C and medigoxin; r) corticosteroids selected from beclomethasone, betamethasone, budesonide, cortisone acetate, desoxymethasone, dexamethasone, fludrocortisone acetate, flunisolide, fluocortolone, fluticasone propionate, hydrocortisone, methylprednisolone, prednisolone, prednisone and triamcinolone; s) diuretics selected from acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorothiazide, chlorthalidone, ethacrynic acid, frusemide, metolazone, spironolactone and triamterene; t) gastrointestinal compounds selected from bisacodyl, cimetidine, cisapride, diphenoxylate HCl, domperidone, famotidine, lanosprazole, loperamide, mesalazine, nizatidine, omeprazole, ondansetron HCl, pantoprazole, rabeprazole sodium, ranitidine HCl and sulphasalazine; u) histamine H_1- and H_2-receptor antagonists selected from acrivastine, astemizole, chlorpheniramine, cinnarizine, cetrizine, clemastine fumarate, cyclizine, cyproheptadine HCl, dexchlorpheniramine, dimenhydrinate, fexofenadine, flunarizine HCl, loratadine, meclizine HCl, oxatomide, and terfenadine; v) keratolytic agents selected from acetretin, calciprotriene, calcifediol, calcitriol, cholecalciferol, ergocalciferol, etretinate, retinoids, targretin, and tazarotene; w) lipid regulating/hypolipidemic agents selected from atorvastatin, bezafibrate, cerivastatin, ciprofibrate, clofibrate, fenofibrate, fluvastatin, gemfibrozil, hesperetin, lovastatin, pravastatin, probucol, and simvastatin; x) opioid analgesics selected from codeine, dextropropoxyphene, diamorphine, dihydrocodeine, fentanyl, meptazinol, methadone, morphine, nalbuphine and pentazocine; and y) nutraceutical agents selected from calcitriol, carotenes, chrysin,

dihydrotachysterol, flavonoids, hesperitin, jasmonates, lipoic acid, lutein, lycopene, essential fatty acids, non-essential fatty acids, naringenin, phytonadiol, quercetin, vitamins including vitamin A, vitamin B2, vitamin D and derivatives, vitamin E, and vitamin K, coenzyme Q10 (ubiquinone), plant extracts, and minerals.

[94] In some embodiments the at least one active ingredient is selected from the group consisting of flavours, flavour precursors, aromas, aroma precursors, taste enhancers, beeswax, salts, sugars, amino-acids, polysaccharides, enzymes, peptides, proteins or carbohydrates, food supplements, food additives, hormones, bacteria, plant extracts, medicaments, drugs, pharmaceuticals, bio-pharmaceutical, biosimilars, vaccines, viral vectors, antigens, antibodies (Abs), peptibodies, antibody-drug-conjugates (ADCs), nutrients, chemicals for agro-chemical or cosmetic applications, carotenoids, vitamins, antioxidants or nutraceuticals selected from the group comprising of lutein, lutein esters, [beta]-carotene, tocopherol, tocopherol acetate, tocotrienol, lycopene, Co-Q10, flax seed oil, fish oil, omega-3 oils, omega-6 oils, DHA, EPA, arachidonic-rich oils, LCPUFA oils, menthol, mint oil, lipoic acid, vitamins, polyphenols and their glycosides, ester and/or sulphate conjugates, isoflavones, flavonols, flavanones and their glycosides such as hesperidin, flavan 3-ols comprising catechin monomers and their gallate esters such as epigallocatechin gallate and their procyanidin oligomers, vitamin C, vitamin C palmitate, vitamin A, vitamin B12, vitamin D, CC-and [gamma]-polyunsaturated fatty acids, phytosterols, esterified phytosterol, non-esterified phytosterol, zeaxanthine, caffeine and a combination thereof.

[95] The present emulsions are suitable for use in the food industry such as ice-cream sector, mayonnaise sector or margarine sector. As contemplated herein, the term "food industry" includes but is not limited to nutritional, functional and nutraceutical foods. Those of skill in the art would be familiar with the particular food industry requirements and be able to adapt the technical teachings of the present oil-in-water emulsions and methods as needed for the industry. In some embodiments the oil-in-water emulsion comprises food acceptable excipients, additives or ingredients.

[96] In some embodiments there is provided an oil-in-water emulsion for used in the food industry.

[97] In some embodiments there is provided an oil-in-water emulsion for use in making an ice-cream product.

[98] In some embodiments there is provided an oil-in-water emulsion for use in making mayonnaise.

[99] In some embodiments there is provided an oil-in-water emulsion for use in making margarine.

[100] In some embodiments there is provided an oil-in-water emulsion for use in functional foods such as probiotics, pre-biotics and nutraceuticals.

[101] The amount of the active ingredient is higher than 0.00001% of the total weight of the emulsion. Preferably it is higher than 0.00003%, more preferably higher than 0.0001%, and even

more preferably higher than 0.001% of the total weight of the emulsion. The amount of the active ingredient is comprised between 0.00001 and 50 % of the total weight of the emulsion. It is also possible to have an amount of active ingredient comprised between 0.00001 and 40 %. The amount of the active ingredient is lower than 50% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 40% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 30% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 20% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 10% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 5% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 2.5% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 2% of the total weight of the emulsion. Preferably, the amount of active ingredient is lower than 1% of the total weight of the emulsion. Any combination of the lower and upper range is comprised in the scope of the present invention. The amount of the active ingredient can be given in wt% or mol% of the total weight or molarity of the emulsion or the total volume of the emulsion.

[102] In some embodiments, the amount of active ingredient is 10% of the total weight of the emulsion. Preferably, the amount of active ingredient is 7.5% of the total weight of the emulsion. Preferably, the amount of active ingredient is 5% of the total weight of the emulsion. Preferably, the amount of active ingredient is 2.5% of the total weight of the emulsion. Preferably, the amount of active ingredient is 1% of the total weight of the emulsion.

[103] As used in the specification and claims, the term "emulsion" refers to the sum of masses of the hydrophilic phase (e.g. water), the lipophilic phase (e.g. oil or triglyceride) and where stated ingredients dissolved therein.

[104] In some embodiments, the substance used for formation of the particles is liquid at room temperature.

[105] In some embodiments, the substance used for formation of the particles is solid at room temperature.

[106] As used herein the term "room temperature" means a comfortable ambient temperature, generally taken as between about 20°C and 25°C (between about 68 and 77°F) with excursions between 10 and 30°C (between about 59 and 86°F) being contemplate, provided the mean kinetic temperature does not exceed 25°C (77°F).

[107] As used in the specification and claims, the term "triglyceride oil" or "triglyceride" refers to esters in which three molecules of one or more different fatty acids are linked to a glycerol. They are named according to the fatty acid components; *e.g.,* tristearin contains three molecules of stearic acid, and oleodistearin, one of oleic acid and two of stearic acid. By way of example triglycerides include at least one of tricaprin (C_{10}TG), trilaurin (C_{12}TG), trimyristin (C_{14}TG), tripalmitin (C_{16}TG) and tristearin (C_{18}TG), esters or mixtures thereof. In some embodiments the composition of the present invention comprises at least one of tricaprin (C_{10}TG), trilaurin (C_{12}TG),

trimyristin (C_{14}TG), tripalmitin (C_{16}TG) and tristearin (C_{18}TG) or mixtures thereof. (See Table 1 and Examples). Furthermore, different natural occurring mixtures of triglycerides can be used, for example – coconut oil represents mixture of triglycerides with about 47 % lauric chains, 16 % myristic chains, 10 % palmitic chains, 3% stearic chains, 7% caprylic chains, 8% decanoic chains, 7 % oleic chains and 2 % linoleic chains. The term "triglyceride oil" also include cottonseed oil, flaxseed oil, almond oil, safflower oil, palm oil, sunflower oil, avocado oil, soybean oil, corn oil, canola oil, grapeseed oil, rapeseed oil, hazelnut oil, linseed oil, brazil nut oil, peanut oil, rice bran oil, hemp seed oil, olive oil, sesame oil, peanut oil, palm kernel oil, cocoa butter, butter, lard, tallow, medium chain triglyceride oil, long chain triglyceride oil.

Emulsion preparation

[108] Emulsions can be prepared using different known methods.

[109] In some embodiments, hand-shaking of the lipophilic surfactants and the hydrophilic surfactant with the oil component such as triglyceride, can form the initial emulsion.

[110] In some embodiments, the preparation of the initial emulsion is mechanically stirred or injected via applied pressure, e.g. via membrane, valve or capillary.

[111] In some embodiments, the initial emulsion consists of oil drops or droplets, dispersed in water in the presence of at lipophilic surfactant and hydrophilic surfactant and could be prepared by any other method, including membrane emulsification, high pressure homogenization, rotor-stator homogenization, stirred vessels, magnetic or non-magnetic stirring devices (see Examples).

[112] In some embodiments, the initial emulsion consists of oil droplets with different shape and size such as with diameter greater than about 0.5 μm, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8 μm, 9 μm, 10 μm, 20 μm, 30 μm, 40 μm, 50 μm or greater. The oil droplets in the initial emulsion can be of any shape.

[113] In some embodiments, the initial emulsion consists of oil droplets with specific shape and size such as with diameter greater than about 0.5 μm, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8 μm, 9 μm, 10 μm or greater such as 20 μm, 30 μm, 40 μm, 50 μm or greater. The oil droplets in the initial emulsion can be of any shape.

[114] In some embodiments when preparing emulsions with compounds such as active ingredients, which are solid at room temperature, these active ingredients can be melted prior to the initial emulsion formation and maintained at temperature sufficiently high to avoid liquid-solid status transition during emulsification.

[115] In some embodiments, the temperature is not higher than about 10^0C relative to the melting point of the selected oil and surfactants.

[116] In some embodiments, the temperature is not higher than about 10^0C relative to the melting point of the selected triglyceride and surfactants.

[117] In some embodiments, the temperature is not higher than about 7.5^{0}C relative to the melting point of the selected oil and/or surfactants. In some embodiments, the temperature is not higher than about 7.5^{0}C relative to the melting point of the selected triglyceride and surfactants. In some embodiments, the temperature is not higher than about 5^{0}C relative to the melting point of the selected oil and surfactants. In some embodiments, the temperature is not higher than about 5^{0}C relative to the melting point of the selected triglyceride and surfactants. In some embodiments, the temperature is not higher than about 2.5^{0}C relative to the melting point of the selected oil and surfactants. In some embodiments, the temperature is not higher than about 2.5^{0}C relative to the melting point of the selected triglyceride and surfactants. In some embodiments, the temperature is not higher than about 1^{0}C relative to the melting point of the selected oil and surfactants. In some embodiments, the temperature is not higher than about 1^{0}C relative to the melting point of the selected triglyceride and surfactants. In some embodiments, the temperature is not higher than about 0.5^{0}C relative to the melting point of the selected oil and surfactants. In some embodiments, the temperature is not higher than about 0.5^{0}C relative to the melting point of the selected triglyceride and surfactants. In some embodiments, the temperature is not higher than about 0.1^{0}C relative to the melting point of the selected oil and surfactants. In some embodiments, the temperature is not higher than about 0.1^{0}C relative to the melting point of the selected triglyceride and surfactants.

[118] In some embodiments, the temperature is not higher than about 10^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 10^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 7.5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 7.5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 2.5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 2.5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 1^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 1^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 0.5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 0.5^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 0.1^{0}C relative to the melting point of the selected active ingredient. In some embodiments, the temperature is not higher than about 0.1^{0}C relative to the melting point of the selected active ingredient.

[119] In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride oil is tricaprin (C_{10}TG). In some embodiments there is provided an oil-in-water

emulsion wherein the triglyceride oil is trilaurin ($C_{12}TG$). In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride oil is trimyristin ($C_{14}TG$). In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride oil is tripalmitin ($C_{16}TG$). In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride oil is tristearin ($C_{18}TG$).

[120] In some embodiments there is provided an oil-in-water emulsion wherein the triglyceride oil is selected from the group consisting of tricaprin ($C_{10}TG$), trilaurin ($C_{12}TG$), trimyristin ($C_{14}TG$), tripalmitin ($C_{16}TG$) and tristearin ($C_{18}TG$) or mixtures thereof or mixture of triacylglycerols containing various combinations of C10, C12, C14, C16 and C18 fatty chains.

[121] In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the lipophilic surfactant is between about 0.01% and about 2%, between about 0.025% and about 1.9%, between about 0.05% and about 1.8%, between about 0.1% and about 1.5%, between about 0.15% and about 1.25%, between about 0.2% and about 1%, between about 0.4% and about 0.8% of the total weight of the emulsion, and wherein the concentration of the hydrophilic surfactant is between about 0.1% and about 5%, between 0.2% and about 4.5%, between about 0.3% and about 4%, between about 0.4 and about 3.5%, between about 0.5% and about 3%, between about 0.6% and about 2.5%, between about 0.7% and about 2%, between about 0.8% and about 1.5%, between about 0.9% and about 1% of the weight of the total emulsion. In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the lipophilic surfactant is 0.7%. In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the lipophilic surfactant is 2.9%.

[122] In some embodiments there is provided an oil-in-water emulsion wherein the concentration of lipophilic surfactant is about 1.5% of the weight of the total emulsion and the concentration of the hydrophilic surfactant is about 1% of the weight of the total emulsion.

[123] In at least one embodiment, the concentration of the lipophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[124] In at least one embodiment, the concentration of the hydrophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[125] In some embodiments there is provided an oil-in-water emulsion wherein the lipophilic surfactant is at least one lipophilic surfactant selected from the group consisting of sorbitan monoalkylate (C_nSorb) where *n* is between 12 and 18; polyoxyethylene glycol hexadecyl ether ($C_{16}EO_2$), polyoxyethylene glycol stearyl ether ($C_{18}EO_2$), polyethylene glycol monooleyl ($C_{18:1}EO_2$), oleyl phosphate, oleyl acetate and 1-oleoyl-*rac*-glycerol (monoolein) or mixtures thereof.

[126] In some embodiments there is provided an oil-in-water emulsion wherein the hydrophilic surfactant is at least one hydrophilic surfactant selected from the group consisting of:

nonionic polyoxyethylene alkyl ether (C_nEO_m), polyoxyethylene sorbitan monoalkylate ($C_nSorbEO_m$) where *n* is between 12 and 40 and where *m* is between 20 and 50; polyethylene glycol monooleyl ether ($C_{18:1}EO_m$) where *m* is between 7 and 20; polyoxyethylene octyl phenyl ether; anionic sodium dodecyl sulfate (SDS); sodium lauryl ether sulfate (SLES); cationic surfactant cetyltrimethyl ammonium bromide (CTAB); and cocamidopropyl betaine (CAPB) or mixtures thereof.

[127] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion is exposed to or treated to at least two cooling-heating cycles, at least three cooling-heating cycles, at least four cooling-heating cycles, at least five cooling-heating cycles, at least six cooling-heating cycles, at least seven cooling-heating cycles, at least eight cooling-heating cycles or more cooling-heating cycles.

[128] The order in which the emulsions of the present invention are treated can be varied depending on the constituents of the emulsions which have been selected. In some embodiments the emulsion or any of the constituents can be exposed to cooling before heating. In some embodiments the emulsion or any of the constituents can be exposed to heating before cooling.

[129] The time or duration of heating of the emulsion can be varied depending on the constituents of the emulsions which have been selected. In some embodiments the emulsion or constituents of the emulsion can be exposed to heating for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40 or 50 seconds; 1, 2, 5, 10, 15, 20, 30, 40, 50, 60 minutes; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours or more.

[130] The time or duration of cooling of the emulsion can be varied depending on the constituents of the emulsions which have been selected. In some embodiments the emulsion or constituents of the emulsion can be exposed to cooling for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40 or 50 seconds; 1, 2, 5, 10, 15, 20, 30, 40, 50, 60 minutes; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours or more.

[131] As used herein, the terms "cooling", "freezing", "re-cooling" and "re-freezing" are used interchangeably unless specifically stated otherwise or the context of the term dictates differently. In some embodiments of the method of the present invention, the cooling or freezing temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the cooling or freezing temperature of the oil.

[132] As used herein, the terms "heating", "melting", "re-heating" and "re-melting" are used interchangeably unless specifically stated otherwise or the context of the term dictates differently. In some embodiments of the method of the present invention, the heating or melting temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the heating or melting temperature of the oil.

[133] In some embodiments, the melting temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the heating or melting temperature of the triglyceride oil.

[134] In some embodiments, the melting temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of tricaprin (C_{10}TG), trilaurin (C_{12}TG), trimyristin (C_{14}TG), tripalmitin (C_{16}TG) and tristearin (C_{18}TG) or mixtures thereof.

[135] In some embodiments, the freezing temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of tricaprin (C_{10}TG), trilaurin (C_{12}TG), trimyristin (C_{14}TG), tripalmitin (C_{16}TG) and tristearin (C_{18}TG) or mixtures thereof.

[136] In some embodiments there is provided an oil-in-water emulsion wherein the nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm are stabilised with at least one lipophilic surfactant and at least one hydrophilic surfactant. In some embodiment, there is provided an emulsion comprising stabilised nano-sized self-emulsified particulates have a diameter of between about 12 nm to about 480 nm, about 14 nm to about 460 nm, about 16 nm to about 440 nm, about 18 nm to about 420 nm, about 20 nm to about 400 nm, about 22 nm to about 380 nm, about 24 nm to about 360 nm, about 26 nm to about 340 nm, about 28 nm to about 320 nm, 30 nm to about 300nm, about 35 nm to about 280 nm, about 40 nm of about 260nm, about 45 nm to about 240 nm, about 50 nm to about 220, about 55 nm to about 200, about 60 nm to about 180 nm, about 65 nm to about 160 nm, about 70 nm to about 140 nm, about 75 nm to about 120 nm, about 80 nm to about 100 nm.

[137] In some embodiments, there is provided an oil-in-water emulsion wherein the nano-sized self-emulsified particulates with a diameter of about 20 nm.

[138] In some embodiments there is provided an oil-in-water emulsion wherein the centration of the stabilising lipophilic surfactant is between about 0.01% and about 2%, between about 0.025% and about 1.9%, between about 0.05% and about 1.8%, between about 0.1% and about 1.5%, between about 0.15% and about 1.25%, between about 0.2% and about 1%, between about 0.4% and about 0.8% of the total weight of the emulsion, and wherein the concentration of the stabilising hydrophilic surfactant is between about 0.1% and about 5%, between 0.2% and about 4.5%, between about 0.3% and about 4%, between about 0.4 and about 3.5%, between about 0.5% and about 3%, between about 0.6% and about 2.5%, between about 0.7% and about 2%, between about 0.8% and about 1.5%, between about 0.9% and about 1% of the weight of the total emulsion. In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the stabilising lipophilic surfactant is 0.7%. In some embodiments there is provided an oil-in-water emulsion wherein the concentration of the stabilising lipophilic surfactant is 2.9%.

[139] In at least one embodiment, the concentration of the stabilising lipophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[140] In at least one embodiment, the concentration of the stabilising hydrophilic surfactant is higher than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% for example higher than 12 % and specifically up to 20 % of the weight of the total emulsion.

[141] In some embodiments there is provided an oil-in-water emulsion wherein the cooling rate of the cooling-heating cycle is between about 0.01 K min^{-1} and about 1000 K min^{-1}, between about 0.01 K min^{-1} and about 50 K min^{-1}, between about 0.025 K min^{-1} and about 45 K min^{-1}, between about 0.05 K min^{-1} and about 40 K min^{-1}, between about 0.075 K min^{-1} and about 35 K min^{-1}, between about 0.1 K min^{-1} and about 30 K min^{-1}, between about 0.125 K min^{-1} and about 25 K min^{-1}, between about 0.2 K min^{-1} and about 20 K min^{-1}, between about 0.4 K min^{-1} and about 15 K min^{-1}, between about 0.5 K min^{-1} and about 10 K min^{-1}, between about 0.75 K min^{-1} and about 7.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 2.5 K min^{-1}, between about 1.5 K min^{-1} and about 2 K min^{-1}. In some embodiments the cooling rate is about 1 K min^{-1}.

[142] In some embodiments there is provided an oil-in-water emulsion wherein the heating rate of the cooling-heating cycle is between about 0.01 K min^{-1} and about 10 K min^{-1}, between about 0.025 K min^{-1} and about 9.5 K min^{-1}, between about 0.05 K min^{-1} and about 9 K min^{-1}, between about 0.075 K min^{-1} and about 8.5 K min^{-1}, between about 0.1 K min^{-1} and about 8 K min^{-1}, between about 0.125 K min^{-1} and about 7.5 K min^{-1}, between about 0.2 K min^{-1} and about 7 K min^{-1}, between about 0.4 K min^{-1} and about 6.5 K min^{-1}, between about 0.5 K min^{-1} and about 6 K min^{-1}, between about 0.75 K min^{-1} and about 5.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 4.5 K min^{-1}, between about 1.5 K min^{-1} and about 4 K min^{-1}, between about 2 K min^{-1} and about 3 K min^{-1}. In some embodiments, the heating rate of the cooling-heating cycle is about 1 K min^{-1}.

[143] In some embodiments there is provided an oil-in-water emulsion comprising mixtures of triglycerides. In some embodiments the triglyceride is at least one selected from the group consisting of tripalmitin, trimyristin, trilaurin, coconut oil or other vegetable oil. In some embodiments the coconut oil is a mixture of triglycerides with about 47 % lauric chains, 16 % myristic chains, 10 % palmitic chains, 3% stearic chains, 7% caprylic chains, 8% decanoic chains, 7 % oleic chains and 2 % linoleic chains. In some embodiments the triglyceride oil can be selected from cottonseed oil, flaxseed oil, almond oil, safflower oil, palm oil, sunflower oil, avocado oil, soybean oil, corn oil, canola oil, grapeseed oil, rapeseed oil, hazelnut oil, linseed oil, brazil nut oil, peanut oil, rice bran oil, hemp seed oil, olive oil, sesame oil, peanut oil, palm kernel oil, cocoa butter, butter, lard, tallow, medium chain triglyceride oil, long chain triglyceride oil.

[144] In some embodiments, an oil-in-water emulsion containing mixtures of other oils, such as diglycerides (diacylglycerols), mono-glycerides (monoacylglycerols), phospholipids, lysolipids. In some embodiments, such mixtures are used as lipid drug delivery systems.

[145] In some embodiments the oil in the oil-in-water emulsion is an excipient for dissolving, dispersing, or otherwise delivering an active compound, though the oil can be an active ingredient itself.

[146] In some embodiments there is provided an oil-in-water emulsion wherein the active ingredient is at least one selected from the group consisting of flavours, flavour precursors, aromas, aroma precursors, taste enhancers, salts, sugars, amino-acids, polysaccharides, enzymes, peptides, proteins or carbohydrates, food supplements, food additives, hormones, bacteria, plant extracts, medicaments, drugs, pharmaceuticals, bio-pharmaceutical, biosimilars, vaccines, antigens, antibodies (Abs), peptibodies, antibody-drug-conjugates (ADCs), nutrients, chemicals for agro-chemical or cosmetic applications, carotenoids, vitamins, antioxidants or nutraceuticals selected from the group comprising of lutein, lutein esters, [beta]-carotene, tocopherol, tocopherol acetate, tocotrienol, lycopene, Co-Q10, flax seed oil, fish oil, omega-3 oils, omega-6 oils, DHA, EPA, arachidonic-rich oils, LCPUFA oils, menthol, mint oil, lipoic acid, vitamins, polyphenols and their glycosides, ester and/or sulphate conjugates, isoflavones, flavonols, flavanones and their glycosides such as hesperidin, flavan 3-ols comprising catechin monomers and their gallate esters such as epigallocatechin gallate and their procyanidin oligomers, vitamin C, vitamin C palmitate, vitamin A, vitamin B12, vitamin D, CC-and [gamma]-polyunsaturated fatty acids, phytosterols, esterified phytosterol, non-esterified phytosterol, zeaxanthine, caffeine and a combination thereof.

[147] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion is dried in a powder form. By way of example only, small angle X-ray scattering and Cryo-TEM can show that active ingredients and structure of the particulates of oil-in-water emulsion of the invention is reconstituted when the dried emulsion is reconstituted by addition of water.

[148] In some embodiments there is provided an oil-in-water emulsion wherein the emulsion is a starting material, an intermediate product or an additive to a final product.

[149] In some embodiments there is provided an oil-in-water emulsion wherein the starting material, the intermediate product or additive to a final product is a pharmaceutical, agro-chemical, consumer, food supplement or cosmetic starting material, the intermediate product or additive to a final product.

[150] In some embodiments there is provided an oil-in-water emulsion wherein the starting material, the intermediate product or additive to a final product has an increased shelf-life.

[151] In some embodiments there is provided an oil-in-water emulsion for use in enhancing stability, protection against chemical or enzymatic degradation or oxidation of an active ingredient in the oil-in-water emulsion.

[152] In some embodiments there is provided an oil-in-water emulsion for use in enhancing bioavailability, bioaccessibility or absorption of active ingredient during digestion.

[153] In some embodiments there is provided an oil-in-water emulsion for use in controlled release, burst release, or sustained release of an active ingredient during consumption or digestion.

[154] In some embodiments there is provided an oil-in-water emulsion for use in controlled release of aroma or flavour, burst release of aroma or flavour, or sustained release of aroma or flavour to create new or improved sensory or taste properties.

[155] In some embodiments there is provided an oil-in-water emulsion for use in creating different taste, different texture, mouth feel, mouth coating, or creaminess sensation.

[156] In some embodiments there is provided an oil-in-water emulsion for use in taste masking, off taste masking, flavour masking, off flavour masking, taste modulation or flavour modulation.

[157] Here we also disclose in one aspect a pharmaceutical combination comprising the oil-in-water emulsion of the present invention and at least one further pharmaceutically acceptable surfactant, excipient or additive.

[158] In some embodiments the pharmaceutical combination comprises the oil-in-water emulsion of the present invention and at least one or more pharmaceutically acceptable excipients or additive.

[159] As used herein, a "pharmaceutically acceptable excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatine, vegetable oils and polyethylene glycols.

[160] In some embodiments the pharmaceutical combination comprises a therapeutically active ingredient. In some embodiments the therapeutically active ingredient is an anti-cancer agent. In some embodiments the anti-cancer agent is a chemotherapy agent. In some embodiments the anti-cancer agent is a biotherapeutic agent such as an antibody (Ab) or a functional derivative of an Ab. In some embodiments the active ingredient is an antimicrobial agent. In some embodiments the antimicrobial agent is an antibacterial agent such as an antibiotic.

[161] In some embodiments there is provided a pharmaceutical combination for use in medicine.

[162] Here we also describe a low-energy method which leads to oil-in-water nano-emulsions, containing particulates with a diameter range between about 10 nm to about 500 nm. The method requires a combination of hydrophilic surfactants and lipophilic surfactants and controlled variation of the emulsion temperature around melting temperature of the oil. In some embodiments, the melting temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of the oil.

[163] In some embodiments, the freezing temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of the oil.

[164] According to one aspect of the present invention, there is provided a method for producing an oil-in-water emulsion according to the present invention, wherein the method comprising the steps of:

a. preparing an initial emulsion of a lipophilic phase in a hydrophilic phase to form oil droplets of a diameter greater than 1 μm in the presence of at least one lipophilic surfactant and at least one hydrophilic surfactant; and
b. treating the initial emulsion to form an oil-in-water emulsion comprising nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm.

[165] In some embodiments of the method of the present invention, wherein the initial emulsion comprises an intermediate metastable phase.

[166] In some embodiments of the method of the present invention, the initial emulsion is subjected to at least one cooling cycle.

[167] In some embodiments of the method of the present invention, the oil is selected from the group consisting of vegetable oils, triglyceride oils, essential oils, flavouring oils, esters, alkanes, paraffins, waxes, diglycerides, phospholipids and mixtures thereof.

[168] In some embodiments of the method of the present invention, the oil is triglyceride oil.

[169] In some embodiments of the present invention, the method requires usage of hydrophilic and/or lipophilic surfactant and controlled variation of the emulsion temperature around the cooling-heating or melting-freezing temperature of the triglyceride.

[170] In some embodiments of the method of the present invention, the initial emulsion is treated by at least two cooling-heating cycles, at least three cooling-heating cycles, at least four cooling-heating cycles, at least five cooling-heating cycles, at least six cooling-heating cycles, at least seven cooling-heating cycles, at least eight cooling-heating cycles or more cooling-heating cycles.

[171] In some embodiments of the method of the present invention, the heating temperature is not more than about 10^0C, 9^0C, 8^0C, 7^0C, 6^0C, 5^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C above the melting temperature of the triglyceride.

[172] In some embodiments of the method of the present invention, the cooling temperature is not more than about 100^0C, 90^0C, 80^0C, 70^0C, 60^0C, 50^0C, 40^0C, 30^0C, 20^0C, 10^0C, 4.5^0C, 4^0C, 3.5^0C, 3^0C, 2.5^0C, 2^0C, 1.5^0C, 1^0C, 0.5^0C, 0.25^0C, 0.15^0C, 0.1^0C below the freezing temperature of the triglyceride.

[173] In some embodiments of the method of the present invention, the nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm are stabilised. In some embodiments of the method of the present invention, the stabilised nano-sized self-emulsified particulates have a diameter of between about 12 nm to about 280 nm, about 14 nm to about 260 nm, about 16 nm to about 240 nm, about 18 nm to about 220 nm, about 20 nm to about 200 nm, about 22 nm to about 180 nm, about 24 nm to about 160 nm, about 26 nm to about 140 nm, about 28 nm to about 120 nm, 30 nm to about 100nm, about 35 nm to about 80 nm, about 40 nm of about 70 nm, about 45 nm to about 65 nm. In some embodiments, of the method of the

present invention, the stabilised nano-sized self-emulsified particulates have a diameter of about 20 nm.

[174] In some embodiments of the method of the present invention, the cooling rate is between about 0.01 K min^{-1} and about 1000 K min^{-1}, between about 0.01 K min^{-1} and about 50 K min^{-1}, between about 0.025 K min^{-1} and about 45 K min^{-1}, between about 0.05 K min^{-1} and about 40 K min^{-1}, between about 0.075 K min^{-1} and about 35 K min^{-1}, between about 0.1 K min^{-1} and about 30 K min^{-1}, between about 0.125 K min^{-1} and about 25 K min^{-1}, between about 0.2 K min^{-1} and about 20 K min^{-1}, between about 0.4 K min^{-1} and about 15 K min^{-1}, between about 0.5 K min^{-1} and about 10 K min^{-1}, between about 0.75 K min^{-1} and about 7.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 2.5 K min^{-1}, between about 1.5 K min^{-1} and about 2 K min^{-1}. In some embodiments the cooling rate is about 1 K min^{-1}.

[175] In some embodiments of the method of the present invention, the heating rate is between about 0.01 K min^{-1} and about 10 K min^{-1}, between about 0.025 K min^{-1} and about 9.5 K min^{-1}, between about 0.05 K min^{-1} and about 9 K min^{-1}, between about 0.075 K min^{-1} and about 8.5 K min^{-1}, between about 0.1 K min^{-1} and about 8 K min^{-1}, between about 0.125 K min^{-1} and about 7.5 K min^{-1}, between about 0.2 K min^{-1} and about 7 K min^{-1}, between about 0.4 K min^{-1} and about 6.5 K min^{-1}, between about 0.5 K min^{-1} and about 6 K min^{-1}, between about 0.75 K min^{-1} and about 5.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 4.5 K min^{-1}, between about 1.5 K min^{-1} and about 4 K min^{-1}, between about 2 K min^{-1} and about 3 K min^{-1}. In some embodiments, the heating rate of the cooling-heating cycle is about 1 K min^{-1}.

[176] In order that the invention may be readily understood and put into practical effect, particular embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

[177] Triglycerides used in the current invention are purchased from TCI, Alfa Aesar or Sigma-Aldrich and have analytical purity, ≥ 80%, see Table 1. The surfactants used are presented in Table 2.

[178] After emulsion preparation, samples were placed in glass capillaries – 50 mm long, 1 or 2 mm wide and 0.10 mm high. The capillaries were put in a thermostated vessel, consisting of a metal plate with water circulating through it. The vessel is connected to a cryo-thermostate (Julabo CF30), allowing high precision temperature control (accuracy ± 0.2 °C).

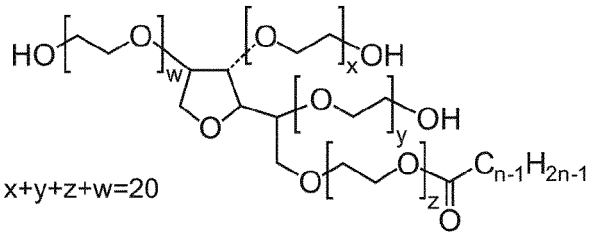
[179] During the cooling/heating of the emulsions a microscope AxioImager.M2m (Zeiss, Germany) was used in transmitted white, polarized light. The microscopes were equipped with λ plate, set at 45° in between the analyzer and the polarizer. The observations were held by the means of long-distance objective with 20x, 50x or 100x (x = times) magnification. The size of the drops

and particles was determined from the microscopic images in which the drops had diameter > 1 μm. The mean volume-surface diameter was determined from the relation:

[180] For determination of the drop sizes in samples with drops smaller than 1 μm the dynamic light scattering method was used. These measurements were performed at 30 °C with 4700C system (Malvern Instruments, U.K.), equipped with a solid state laser, operating at 514 nm. Multimodal software was used for analysis of the autocorrelation function of the light scattered by the emulsion.

[181] Table 1. List of the triglycerides used. The values for melting points are stated as provided by the producers.

Chemical name	Chemical formula	Abbreviation	Producer	Purity	Melting point, T_m,°C
1,2,3-propanetriyl tridecanoate (tricaprin)	$C_{33}H_{62}O_6$	$C_{10}TG$	TCI Chemicals	> 98%	31 - 35
1,2,3-propanetriyl tridodecanoate (trilaurin)	$C_{39}H_{74}O_6$	$C_{12}TG$	Alfa Aesar	≥ 99%	47
1,2,3-propanetriyl tritetradecanoate (trimyristin)	$C_{45}H_{86}O_6$	$C_{14}TG$	Sigma Aldrich	≥ 99%	56 - 57
			TCI Chemicals	> 95%	55 - 60
1,2,3-propanetriul trihexadecanoate (tripalmitin)	$C_{51}H_{98}O_6$	$C_{16}TG$	TCI Chemicals	> 85%	64 - 68
1,2,3-propanetriyl trioctadecanoate (tristearin)	$C_{57}H_{110}O_6$	$C_{18}TG$	TCI Chemicals	> 80%	66 - 74

	Non-ionic surfactant (trade name/product No)	Number of C atoms, n	Number of EO groups, m	Producer	HLB	Structural formula
Polyoxyethylene alkyl ethers C_nEO_m	Brij 30	12	4	Sigma - Aldrich	9	$C_nH_{2n+1}(OCH_2CH_2)_mOH$
	Brij 52	16	2		5	
	Brij 72	18	2		4.9	
	Brij C10	16	10		12	
	Brij S10	18	10		12	
	Brij 35	12	23		16	
	Brij 58	16	20		15.7	
	Brij S20	18	20		15.3	
	Lutensol AT25	16-18	25	BASF	16	
	Lutensol AT50	16-18	50		18	
	Nonidac 22-30	22	30	Sasol	16	
	Performathox 480	20-40	40	New Phase Tech.	16	
Polyoxyethylene Sorbitan monoalkylate $C_nSorbEO_m$	Tween 20	12	20	Sigma - Aldrich	16.7	 x+y+z+w=20
	Tween 40	16	20		15.5	
	Tween 60	18	20		14.9	
	Tween 80	18 with double bond	20		15.0	
Polyethylene glycol monooleyl ethers $C_{18:1}EO_m$	P0711	18 with double bond	2	TCI Chemicals	4	$C_{18}H_{35}(OCH_2CH_2)_mOH$
	P0713		7		10.7	
	P0714		10		12.4	
	P0715		20		15	
	Span 20	12	-	Merck	8.6	

Sorbitan monoalkylates SorbC_n	Span 40	16			TCI Chemicals	6.7			
	Span 60	18			Sigma-Aldrich	4.7			
	Span 80	18 with double bond			Sigma-Aldrich	4.3			
Polyoxyethylene octyl phenyl ether	Triton X100	octyl phenyl	9.5		Merck	13.5	*t*-Oct-C_6H_4-$(OCH_2CH_2)_mOH$		
	Substances used as non-ionic surfactant (name /product No)	**Number of C atoms, n**	**Number of EO groups, m**		**Producer**	**Purity**	**Structural formula**		
Monoolein	1-oleoyl-*rac*-glycerol	18 with double bond	-		Sigma-Aldrich	~ 40 %	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOCH_2CHOHCH_2OH$		
Oleyl acetate	A0934	18 with double bond	-		TCI Chemicals	> 60 %	$H_3COOCH_2(CH_2)_6CH_2{=}CH_2(CH_2)_6CH_3$		
Monopalmitin	G0083	16	-		TCI Chemicals	> 95 %	$HOCH_2CH(OH)CH_2OCO(CH_2)_{14}CH_3$		
Monostearin	G0085	18	-		TCI Chemicals	> 60 %	$CH_3(CH_2)_{16}COOCH_2CH(OH)CH_2OH$		
	Ionic surfactant	**Number of C atoms, n**	**Hydrophilic head group**		**Producer**	**Purity**			
Anionic	SDS	12	$SO_4^-Na^+$		Sigma	99 %	$CH_3(CH_2)_{11}OSO_3Na$		
	SLES	12	$(CH_2CH_2O)_2$ SO_4Na^+		BASF	-	$CH_3(CH_2)_{11}(OCH_2CH_2)_nOSO_3Na$		
Cationic	CTAB	16	$N^+(CH_3)_3(Br^-)$		Sigma	> 99 %	$C_{16}H_{33}[N(CH_3)_3]Br$		
Zwitterionic	CAPB	12	-		Plovdiv	40 %	$CH_3(CH_2)_{10}CONHCH_2CH_2CH_2N^+(CH_3)_2CH_2CO_2^-$		

[182] **Example 1 Preparation of nanoemulsions by freeze-thaw cycling of the emulsion**

[183] This example demonstrates the drop-size decrease, illustrated in Figure 1 (a) and (b). 1.5 wt. % Brij S20 and 1 wt. % monopalmitin and monostearin mixture is dispersed in water. $C_{14}TG$ is added to the aqueous phase through membrane emulsification. The triglyceride volume fraction in the prepared emulsion is 0.7 vol. %. The initial drop or droplet size in the prepared emulsion is 20 μm (Figure 1(a)) or 33.5 μm (Figure 1(b)). The emulsion is cooled from 335 K to 278 K in a fridge and then heated back up to 335 K in a water bath – where only the lipid component of the emulsion is solidified. After two consecutive freeze-thaw cycles the drop diameter by volume become 100 nm and after 4 freeze-thaw cycles, it becomes 20 nm. This drop diameter does not change if more freeze-thaw cycles are performed. Depending on the final temperature, the droplets could be liquid or solid.

[184] **Figure 1(a)** Illustrates the drop-size decrease observed with large scale or bulk emulsions. The smallest drop diameter which is reached after 4 consecutive freeze/thaw cycles for emulsion with 0.7 vol. % oil and drops with initial diameter around 20 μm is 20 nm. The emulsion is prepared with $C_{14}TG$ and stabilized by 1.5 wt.% $C_{18}EO_{20}$ and 1 wt.% monopalmitin and monostearin mixture, dispersed in the aqueous phase.

[185] **Figure 1(b)** Illustrates the drop size decrease observed with large scale or bulk emulsions. The smallest drop or droplet diameter which is reached after 4 consecutive freeze/thaw cycles for emulsion with 2.9 vol. % oil and drops with initial diameter around 33.5 μm is 40 nm. The emulsion is prepared with $C_{14}TG$ and stabilized by 1.5 wt.% $C_{18}EO_{20}$ and 1 wt.% monopalmitin and monostearin mixture, dispersed in the aqueous phase.

[186] **Example 2 Preparation of nanoemulsion without freeze-thaw cycling of the emulsion**

[187] This example demonstrates the drop-size decrease observed in emulsions which are stored at 35°C without heating and/or additional cooling. The initial emulsion is prepared by membrane emulsification and drops or droplet have initial diameter of 35 μm. Emulsion contains 2.9 vol. % emulsified $C_{14}TG$ and water phase containing 1.5 wt. % Brij S20 and 1 wt. % monopalmitin and monostearin mixture. The emulsion drops are prepared at temperature of 335 K. After the initial preparation, the emulsion is placed in a glass capillary which is sealed and stored at 35°C. The emulsion is observed under a microscope upon different periods of storage, see Figure 2. Drop disintegration upon storage is observed. The particles that form upon the disintegration have diameter smaller than 1 μm.

[188] **Figure 2** Depicts the particle disintegration observed upon storage of the emulsion. Particle disintegration observed with $C_{14}TG$ sample stored at 35°C. After 19 h at 35°C, the particle volume noticeably increased and the disintegration process has already begun. After 67 h, significant fraction of the particles has already disintegrated. The reason for the lower contrast of this image is the appearance of numerous small droplets which scatter the light illuminating the

sample. These observations are performed in a glass capillary which was stored in a thermostat at 35°C and was observed at certain time intervals, as indicated in the images.

[189] The disclosure illustratively described herein can suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed.

* * *

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WHAT IS CLAIMED:

1. Oil-in-water emulsion wherein oil droplets of a diameter greater than 1 μm exhibit nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm, due to the presence of:
   a. at least one lipophilic surfactant; and/or
   b. at least one hydrophilic surfactant.

2. Oil-in-water emulsion according to claim 1, wherein the oil-in-water emulsion comprises one or more active ingredients.

3. Oil-in-water emulsion according to claim 2, wherein the active ingredient is lipophilic.

4. Oil-in-water emulsion according to any one of claims 2 or 3, wherein the active ingredient is between 0.0001% and 50% of the total weight of the emulsion.

5. Oil-in-water emulsion according to any one of the preceding claims, wherein the emulsion comprises oil droplets having nano-sized self-emulsified particulates comprising:
   i) oil selected from the group consisting of vegetable oils, triglyceride oils, essential oils, flavouring oils, esters, alkanes, paraffins, waxes, diglycerides, phospholipids and mixtures thereof;
   ii) at least one lipophilic surfactant with HLB value (Hydrophilic-Lipophilic Balance) < 10 and/or at least one hydrophilic surfactant, having HLB ≥ 10.

6. Oil-in-water emulsion according to claim 5, wherein the oil is a triglyceride oil.

7. Oil-in-water emulsion according to claim 5 or claim 6, wherein the triglyceride oil is selected from the group consisting of tricaprin (C_{10}TG), trilaurin (C_{12}TG), trimyristin (C_{14}TG), tripalmitin (C_{16}TG) and tristearin (C_{18}TG) or mixtures thereof.

8. Oil-in-water emulsion according to any one of claims 1 to 7, wherein for 1% oil content in the emulsion the preferred concentration of the lipophilic surfactant is between about 0.01% and about 3%, between about 0.025% and about 2.8%, between about 0.05% and about 2.6%, between about 0.1% and about 2.4%, between about 0.2% and about 2%, between about 0.4% and about 2%, between about 0.8% and about 1.8% of the total weight of the emulsion, and wherein the concentration of the hydrophilic surfactant is between about 0.1% and about 5%, between 0.2% and about 4.5%, between about 0.3% and about 4%, between about 0.4 and about 3.5%, between about 0.5% and about 3%, between about 0.6% and about 2.5%, between about 0.7% and about 2%, between about 0.8% and about 1.5%, between about 0.9% and about 1% of the weight of the total emulsion.

9. Oil-in-water emulsion according to claim 8, wherein the concentration of the lipophilic surfactant is about 1.5% of the weight of the total emulsion and the concentration of the hydrophilic surfactant is about 1% of the weight of the total emulsion.

10. Oil-in-water emulsion according to anyone of claim 1 to 9, wherein the lipophilic surfactant is at least one lipophilic surfactant selected from the group consisting of sorbitan monoalkylate (C_nSorb) where *n* is between 12 and 18; polyoxyethylene glycol hexadecyl ether ($C_{16}EO_2$), polyoxyethylene glycol stearyl ether ($C_{18}EO_2$), polyethylene glycol monooleyl ($C_{18:1}EO_2$), oleyl phosphate, oleyl acetate and 1-oleoyl-*rac*-glycerol (monoolein).

11. Oil-in-water emulsion according to claims 1 to 10, wherein the hydrophilic surfactant is at least one hydrophilic surfactant selected from the group consisting of: nonionic polyoxyethylene alkyl ether (C_nEO_m), polyoxyethylene sorbitan monoalkylate (C_nSorbEO_m) where *n* is between 12 and 40 and where *m* is between 20 and 50; polyethylene glycol monooleyl ether ($C_{18:1}EO_m$) where *m* is between 7 and 20; polyoxyethylene octyl phenyl ether; anionic sodium dodecyl sulfate (SDS); sodium lauryl ether sulfate (SLES); cationic surfactant cetyltrimethyl ammonium bromide (CTAB); and cocamidopropyl betaine (CAPB).

12. Oil-in-water emulsion according to any one of claims 1 to 11, wherein the emulsion is exposed to at least two cooling-heating cycles.

13. Oil-in-water emulsion according to any one of claims 1 to 12, wherein the nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm are stabilised with at least one lipophilic surfactant and at least one hydrophilic surfactant.

14. Oil-in-water emulsion according to claim 13, wherein the stabilised nano-sized self-emulsified particulates have a diameter of about 20 nm to 50 nm.

15. Oil-in-water emulsion according to any one of claims 13 or 14, wherein the concentration of the stabilising lipophilic surfactant is between about 0.01% and about 2%.

16. Oil-in-water emulsion according to any one of claims 12 to 15, wherein the cooling rate of the cooling-heating cycle is between about 0.01 K min^{-1} and about 1000 K min^{-1}, between about 0.025 K min^{-1} and about 50 K min^{-1}, between about 0.05 K min^{-1} and about 40 K min^{-1}, between about 0.075 K min^{-1} and about 35 K min^{-1}, between about 0.1 K min^{-1} and about 30 K min^{-1}, between about 0.125 K min^{-1} and about 25 K min^{-1}, between about 0.2 K min^{-1} and about 20 K min^{-1}, between about 0.4 K min^{-1} and about 15 K min^{-1}, between about 0.5 K min^{-1} and about 10 K min^{-1}, between about 0.75 K min^{-1} and about 7.5 K min^{-1}, between about 1 K min^{-1} and about 5 K min^{-1}, between about 1.25 K min^{-1} and about 2.5 K min^{-1}, between about 1.5 K min^{-1} and about 2 K min^{-1}.

17. Oil-in-water emulsion according to any one of claims 12 to 15, wherein the heating rate of the cooling-heating cycle is between about 0.01 K min^{-1} and about 1000 K min^{-1}.

18. Oil-in-water emulsion according to any one of claims 1 to 17, wherein the emulsion comprises trimyristine, $C_{18}EO_{20}$ and $C_{18:1}EO_2$.

19. Oil-in-water emulsion according to any one of claims 2 to 18, wherein the active ingredient is at least one selected from the group consisting of flavours, flavour precursors, aromas, aroma precursors, taste enhancers, beeswax, salts, sugars, amino-acids, polysaccharides, enzymes, peptides, proteins or carbohydrates, food supplements, food additives, hormones, bacteria, plant extracts, medicaments, drugs, pharmaceuticals, antineoplastics (including curcumin and cannabinoids), psychoactives, steroids and modified steroids, sterols (including cholesterol), bio-pharmaceutical, constrained peptides, biosimilars, vaccines, antigens, antibodies (Abs), peptibodies, antibody-drug-conjugates (ADCs), nutrients, chemicals for agro-chemicals (including terpenes), chemicals for cosmetic applications, carotenoids, vitamins, antioxidants or nutraceuticals selected from the group comprising of lutein, lutein esters, [beta]-carotene, tocopherol, tocopherol acetate, tocotrienol, lycopene, Co-Q10, flax seed oil, fish oil, omega-3 oils, omega-6 oils, DHA, EPA, arachidonic-rich oils, LCPUFA oils, menthol, mint oil, lipoic acid, vitamins, polyphenols and their glycosides, ester and/or sulphate conjugates, isoflavones, flavonols, flavanones and their glycosides such as hesperidin, flavan 3-ols comprising catechin monomers and their gallate esters such as epigallocatechin gallate and their procyanidin oligomers, vitamin C, vitamin C palmitate, vitamin A, vitamin B12, vitamin D, CC-and [gamma]-polyunsaturated fatty acids, phytosterols, esterified phytosterol, non-esterified phytosterol, zeaxanthine, caffeine and a combination thereof.

20. Powder and/or lipid nanoparticles wherein the oil-in-water emulsion according to any one of claims 1 to 19 is stabilized and dried in a powder form.

21. Oil-in-water emulsion according to any one of claims 1 to 19, wherein the emulsion is a final product, a starting material, an intermediate product or an additive to a final product.

22. Oil-in-water emulsion according to claim 21 wherein the starting material, the intermediate product or additive to a final product is a pharmaceutical, agro-chemical, consumer, food supplement or cosmetic starting material, the intermediate product or additive to a final product.

23. Oil-in-water emulsion according to any one of claims 1 to 19 for use in enhancing stability, protection against chemical or enzymatic degradation or oxidation of an active ingredient in the oil-in-water emulsion.

24. Oil-in-water emulsion according to any of claims 1 to 19 for use in enhancing bioavailability, bioaccessibility, absorption of active ingredient during digestion,

controlled release, burst release, or sustained release of an active ingredient during consumption or digestion.

25. Oil-in-water emulsion according to any one of claims 1 to 19 for use in controlled release of aroma or flavour, burst release of aroma or flavour, or sustained release of aroma or flavour to create new or improved sensory or taste properties.

26. Oil-in-water emulsion according to any one of claims 1 to 19 for use for creating different taste, different texture, mouth feel, mouth coating, or creaminess sensation.

27. Oil-in-water emulsion according to any one of claims 1 to 19 for use in taste masking, off taste masking, flavour masking, off flavour masking, taste modulation or flavour modulation.

28. A pharmaceutical combination comprising the oil-in-water emulsion according to any one of claims 1 to 19 and at least one further pharmaceutically acceptable surfactant, excipient or additive.

29. Oil-in-water emulsion according to any one of claims 1 to 19 or a pharmaceutical combination according to claim 28, for use in medicine.

30. A method for producing an oil-in-water emulsion according to any one of claims 1 to 19, the method comprising the steps of:
    a. preparing an initial emulsion of a lipophilic phase in a hydrophilic phase to form oil droplets of a diameter greater than 1 μm in the presence of at least one lipophilic surfactant and/or at least one hydrophilic surfactant; and
    b. cooling and/or heating the initial emulsion to form an oil-in-water emulsion comprising nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm.

31. A method according to claim 30, wherein the mixture is exposed to at least two cooling-heating cycles.

32. A method according to anyone of claims 30 or 31, wherein the nano-sized self-emulsified particulates with a diameter in the range of about 10 nm to about 500 nm are stabilised and/or dried in a powder form.

A method according to claim 32, wherein the stabilised nano-sized self-emulsified particulates have a diameter of about 20 nm to 50 nm.

FIG 1. Illustrates the drop size decrease observed with bulk emulsions.

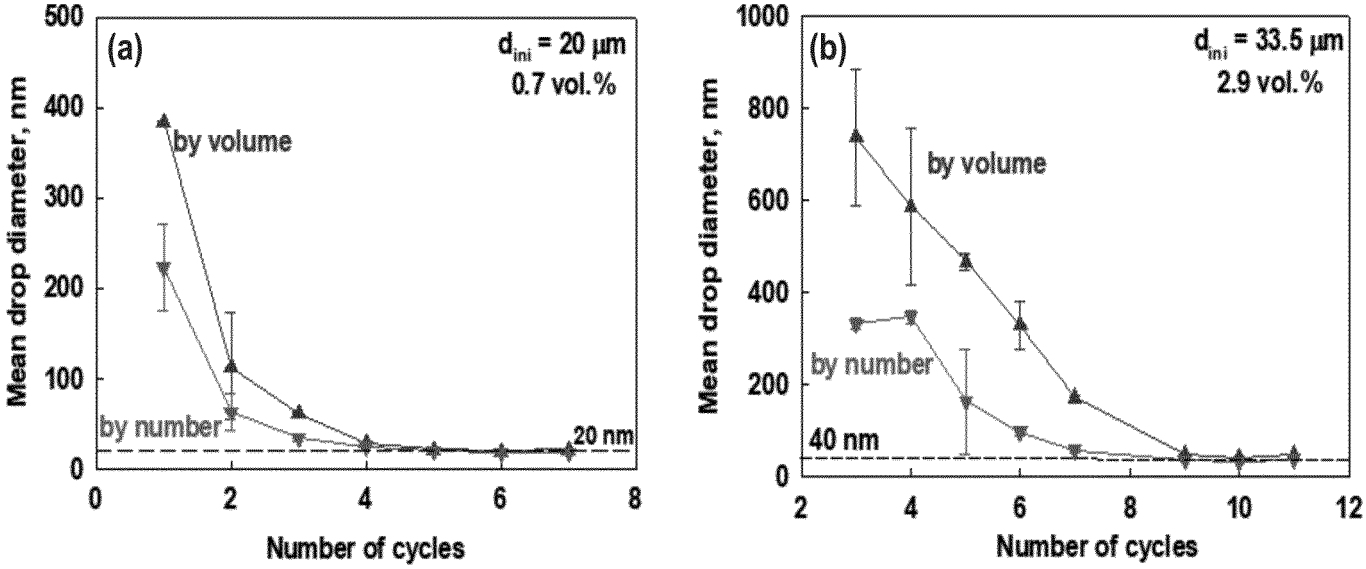
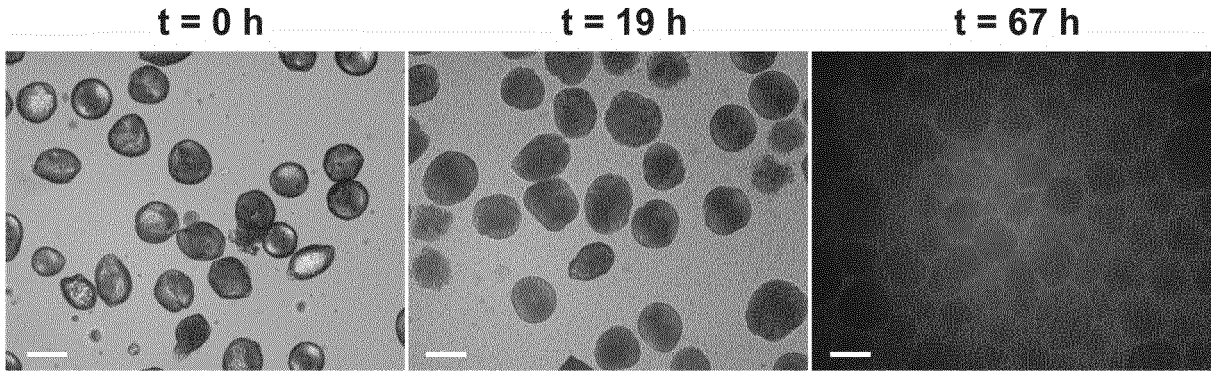


FIG 2. Particle disintegration observed upon storage at 35°C. Scale bars are 50 µm.



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/068018

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01F17/00 B01F17/08 B01F17/42
ADD.

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)
B01F

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)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 598 060 A1 (NESTEC SA [CH]) 23 November 2005 (2005-11-23)	1-29
A	claims 1-14	30-33

X	WO 2007/060177 A1 (NESTEC SA [CH]; SAGALOWICZ LAURENT [CH] ET AL.) 31 May 2007 (2007-05-31)	1-29
A	claims 1-29; figure 9	30-33

A	EP 0 696 452 A1 (CUSI LAB [ES]) 14 February 1996 (1996-02-14) claims 1-18	1-33

A,P	WO 2020/018512 A1 (NANOGEN LAB INC [US]) 23 January 2020 (2020-01-23) claims 1-47	1-33

☐ Further documents are listed in the continuation of Box C.

☒ 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
16 October 2020	26/10/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Richards, Michael

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2020/068018

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		BR PI0510147 A	02-10-2007
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