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(54) Title: CHIA OIL EDIBLE NANOEMULSION

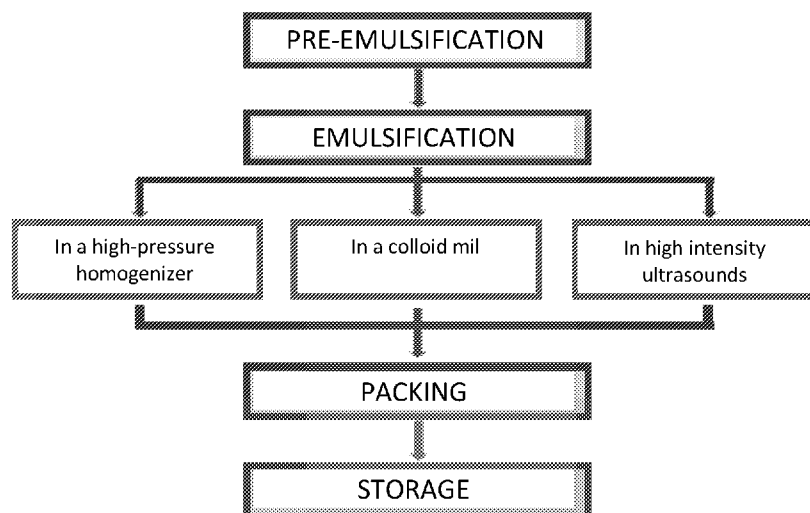


FIGURE 1

(57) **Abstract:** An edible chia oil nanoemulsion, rich in omega-3, with a high physical and oxidative stability, high bioaccessibility of omega-3 fatty acids and preparation methods. In particular, it discloses a chia oil nanoemulsion comprising between 10% and 20% of chia oil (*Salvia hispanica L.*), between 2% and 5% of polysorbate, between 0.5% and 5% of at least one emulsifier other than the polysorbate, between 0.05% and 0.2% of at least one antioxidant and water. In some specific preparations, the chia oil nanoemulsion of this invention may also comprise at least one pH modifier. In other specific preparations, the chia oil nanoemulsion of this invention may also comprise one or more polysaccharides. In other specific preparations, the chia oil nanoemulsion of this invention may also comprise one or more sugars. Also, edible formulations are disclosed, comprising chia oil nanoemulsions and, in particular, transparent drinks and desserts, such as juices and jellies with such nanoemulsions.



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CHIA OIL EDIBLE NANOEMULSION

This invention refers, in general, to a chia oil edible nanoemulsion. In particular, it refers to a chia oil nanoemulsion comprising between 10% and 20% of chia oil (*Salvia hispánica L.*), between 2% and 5% of polysorbate, between 0.5% and 5% of at least one emulsifier other than the polysorbate, between 0.05% and 0.2% of at least one antioxidant and water. In some specific preparations, the chia oil nanoemulsion of this invention may also comprise at least one pH modifier. In other specific preparations, the chia oil nanoemulsion of this invention may also comprise one or more polysaccharides. In other specific preparations, the chia oil nanoemulsion of this invention may also comprise one or more sugars.

INVENTION BACKGROUND INFORMATION

During the latest years, chia oil (also known as chia seed oil or *Salvia Hispánica L.* seeds oil) has become very relevant for its impact on health. The chia seeds oil is a vegetable oil obtained from Chia or *Salvia Hispánica* seeds, which has a high content of omega-3 (α -linolenic acid) polyunsaturated fatty acids. The chia oil is a vegetable source with a higher percentage of α -linolenic acid known so far (62 - 64%), as well as essential fatty acids (82.3%) (α -linolenic and linoleic acids) (Ayerza, 1995). In the literature, it has been reported to make large contributions to cardiovascular health care, as it helps control the cholesterol level in blood, in inflammatory processes, in immune system disorders and it has also antioxidant effects. Therefore, it may be said that the main chia oil attribute is to be an excellent natural source of omega-3 fatty acids.

The chia oil is an alternative for the fish oil as an omega-3 source for those persons who are not fish consumers for any reason, such as vegetarians or persons with certain allergies. On the other hand, it is worth mentioning that the chia oil, as compared with other oils, is more stable to the oxidation of its omega-3 fatty acids, due to natural antioxidants in its seeds and, it is odorless. An additional advantage with respect to fish oil is the absence of cholesterol.

Due to its high omega-3 fatty acid contents, it would be sufficient to have a few chia oil grams a day (such as, a teaspoonful) of raw oil to cover the daily needs of α -linoleic acid.

The inclusion of bioactive lipids—such as the chia oil—in food with high water content (drinks), poses some technological challenges, including the way to carry them in the aqueous phase, their protection against oxidation and during their gastrointestinal transit and the assurance of their its release in the small intestine, where the fatty acids will be absorbed, i.e., its bioaccessibility and bioavailability.

The emulsification technique is broadly used in the food, pharmaceutical and cosmetic industries. In traditional emulsions, the droplet size is between 0.5 and 10 microns. Nanoemulsions, instead, are emulsions whose droplet diameter is lower than 200 nm. The nanoemulsion formulation with a droplet size between 50 and 200 nm (0.05 and 0.2 microns), started to develop more recently and has become increasingly relevant within the frame of nanotechnologies. Nanoemulsions are particularly suitable for the manufacture of nano-encapsulated functional compounds, as they present

better properties than the conventional emulsions (higher kinetic stability, protection from oxidation and higher transparency). Therefore, the most practical way to include relatively high bioactive lipids concentrations (omega-3) in transparent products, would be by the use of nanoemulsions with such small particles so as to avoid light scattering. However, until today no successful results have been reported about the chia oil nanoemulsification.

The nanoemulsions must be compatible and stable in the end product. This implies that they should not alter the product appearance, taste, texture, etc. They should fortify transparent or slightly opaque products, such as drinks (juice, flavored water, drinks for sport athletes, etc.) or jellies, representing a technological challenge as it has to be achieved a nanoemulsion that not only has a very small droplet size (total transparency is achieved for droplet sizes smaller than 50 nm), but also remains stable in the final product.

During recent years, the relevance of the emulsification as a system to deliver certain bioactive lipids as omega-3 fatty acids, in looking for an improvement of their bioaccessibility. The digestion of lipids is an interfacial process, in which the co-lipase cofactor (synthesized in the pancreas and secreted in the duodenum) has to be absorbed in the film which covers the oil droplet (oil-water interface). Thus, the lipase could hydrolyze the triglycerides (TG), releasing fatty acids, which would be absorbed in the small intestine. The interfacial area (dependent on the droplet size) and the interface composition (dependent on the type of emulsifier absorbed) are the main factors controlling the release of fatty acids

during the digestion of lipids. The formation of nanoemulsions generates a huge interfacial area available for the action of lipases, which promotes a maximum release of fatty acids (among which are the omega-3 acids), increasing bioaccessibility. However, no results have been reported so far about the impact of the composition of emulsifiers in the bioaccessibility / bioavailability of the chia oil fatty acids.

In patent: **US 8,460,727 B2**, although the chia oil is mentioned, it discloses that it is obtained by means of a simple and effective micro-encapsulation method using spray drying under special conditions, for obtaining powder products, internationally known as DMFO (*dried microencapsulated fish oil*). The products consist in oil micro-drops (with a drop-let size higher than 500 nm or its equivalent, 0.5 microns) covered by a carbohydrate matrix which protects them against external agents, such as the light, oxygen and temperature. The product disclosed there may not be used in transparent drinks, as it contributes a high level of turbidity and a large amount of carbohydrates, which could not be compatible with this type of drinks. Also, due to their neutral pH, they could not be included in acid drinks.

In the publication: "*Physicochemical characterization and stability of chia oil microencapsulated with sodium caseinate and lactose by spray-drying*". *Powder Technology* 271 (2015) 26 -34, V.Y. Ixtaina & col., chia oil microencapsulated with sodium caseinate and lactose is disclosed, which would be within the scope of considerations similar to those mentioned above.

In patent application: **CA2623903 A1**, emulsions of an

equivalent droplet size higher than 0.2 μm are disclosed. Thus, emulsions for acidic drinks, as disclosed there, may not be deemed as nanoemulsions and are not transparent when they are added to food in general and to transparent drinks, in particular. Also, they present physical instability. The method used for obtaining such emulsions is based on the formation of interfacial multi-layers, as a result of the electrostatic interaction of oppositely charged molecules.

In patent application: **US2015051298 (A1)** oil nanoemulsions are disclosed, which were obtained by a spontaneous emulsification method. As described there, such method requires high concentrations of emulsifiers of a low molecular weight, in particular, polysorbate concentrations higher than 10% and 20%, which have regulatory restrictions. As disclosed there, to achieve nanoemulsions by the spontaneous emulsification method, another oil is required, generally MCT (*medium chain triglycerides*).

In patent application **WO2012/032416** edible emulsions are disclosed, which basically use 2 emulsifiers: vitamin E TPGS & polysorbates. To obtain them, very high concentrations of emulsifiers are required, in particular, concentrations of polysorbates higher than 10% and up to 20%, which present regulatory restrictions. Also, based on the teachings of the application mentioned above, a large amount of carbohydrates is used, which may not be compatible with the drinks and may crystallize. In addition due to the acid pH of many drinks, the sucrose may be inverted.

On the other hand, although patent: **US 8,628,690 B2** mentions nanoemulsions, from its teachings only emulsions

may be obtained. Note, for example, that in its preparation examples the diameter of the droplets is mentioned to be higher than 200 nm, which is not consistent with nanoemulsions. Therefore, the emulsions disclosed in the document mentioned above would not allow obtaining transparency when they are added to drinks.

On the other hand, in the publication "*Chia seed oil-in-water emulsions as potential delivery systems of w-3 fatty acids*", Journal of Food Engineering 162 (2015) 48-55, by Luciana Magdalena Julio and col., no chia oil nanoemulsions are disclosed but only emulsions (note that the droplet sizes obtained are higher than 0.3 μm), which are not transparent when added to drinks. Also, these emulsions are reported to be little stable and, therefore, they would not be commercially applicable.

Thus, there is a specific unsatisfied need for obtaining a chia oil nanoemulsion, as a source of omega 3, which is highly bioavailable and physically stable and stable to oxidation, which could be added to food in general, and to transparent food and drinks in particular.

BRIEF DESCRIPTION OF THE INVENTION

This invention refers, in general, to a chia oil edible nanoemulsion as a source of omega 3 and to its preparation methods. In particular, it refers to a chia oil nanoemulsion comprising between 10% and 20% of chia oil (*Salvia hispanica* L.), between 2% and 5% of polysorbate, between 0.5% and 5% of at least one emulsifier other than the polysorbate, between 0.05% and 0.2% of at least one antioxidant and water. This invention involves the optimization of the formulation and a preparation method to

ensure the following set of properties:

A droplet size lower than 200 nm, which is stable over time.

High resistance to oxidation.

High bioaccessibility of omega-3 fatty acids.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Scheme of the procedure for obtaining chia oil nanoemulsion in the invention.

Figure 2. Droplet distribution size and the prevailing diameter value in nanometers (nm) of the chia oil in the nanoemulsion, in the formulations, based on the invention **F1** to **F7**. A prevailing diameter value lower than 90 nm is noted in all the cases.

Figure 3. Nanoemulsions images based on invention **F1** to **F3**. Nanoemulsions are noted to be translucent.

Figure 4. Droplet distribution size and prevailing diameter value in nanometers (nm) of the chia oil in the nanoemulsion, in the invention **F8** formulation obtained by means of high-intensity ultrasound (**F8 US**) or in a high-pressure homogenizer (**F8 HG**). It is noticed that with both methods it is possible to obtain a nanoemulsion, whose main population has a droplet diameter of 79 nm.

Figure 5. Evolution of the average diameter along the time of ~~such~~ formulations based on **F1** & **F4** invention. It may be noticed that during two months of storage, the emulsions keep stable.

Figure 6. Evolution along the time of the chia oil peroxide

index in the nanoemulsion, based on **F2** invention and the latter added with natural antioxidants (**F10**, **F11** & **F12**). It may be noticed that the combination of natural antioxidants(**F12**) results in a higher oxidative stability.

Figure 7. Fatty acid release profile of the chia oil and of the nanoemulsion based on **F1** invention during *in vitro* duodenal digestion. For comparative purposes, a nanoemulsion containing a single polysorbate emulsifier at 5% (**F9**) is shown. A higher degree of fatty acid release is noticed when the chia oil is emulsified. An increased bioaccessibility of fatty acids can be identified when at least an additional emulsifier is used in addition to the polysorbate.

Figures 8 & 9. Distribution of the chia oil droplet size in nanoemulsion (A) and in apple juice where a nanoemulsion has been added, based on the invention, in a 1:40 proportion along the time (B). A portion of such juice (200 ml) would contribute the daily recommended intake (DRI) of omega 3 (World Health Organization, WHO). The diameter value in nanometers (nm) has been indicated. For comparative purposes, **Figure B** also shows the size distribution of the pure apple juice particles. **Figure 8** shows the results obtained for **F2** nanoemulsion (**L2 5% + PS 5%, pH 3**) and **Figure 9** shows the results of **F3** nanoemulsion. It may be noticed that the populations of approximately 300 nm appearing on juices where the nanoemulsions of the invention were added correspond to the particles in the juice and would not represent a destabilization of the original nanoemulsion, which continues maintaining its size.

Figure 10. Turbidity of the apple juice with different

nanoemulsions added, based on the formulation of invention **F1 (F1M)** & **F3 (F3M)**, diluted 1:40 & 1:200, as measured with a spectrophotometer at a wave length of 600 nm. A portion (200 ml) of such juice added with the dilution of 1:200 of such nanoemulsion would contribute 20% of the daily recommended intake (DRI) of omega 3 (World Health Organization, WHO). For control purposes, the results obtained for the pure apple juice may be obtained (**MAN**). It may be noticed that adding the proportion of 1:200 in the apple juice presents a very low level of turbidity, as compared with the pure juice.

Figure 11. Images of the pure apple juice (**MAN**) added with different nanoemulsions, based on the **F1 (F1M)** & **F3 (F3M)** invention, in a dilution of 1:40 & 1:200. It may be noticed that adding a proportion of 1:200 does not substantially change the juice aspect.

Figures 12 & 13. Distribution of chia oil droplet size in the nanoemulsion (A) and in orange juice added with a nanoemulsion, based on the invention, in a 1:40 proportion along the time (B). The prevailing diameter value in nanometers (nm) has been indicated. For comparative purposes, **Figure B** also shows the size distribution of the pure orange juice particles. **Figure 12** shows the results obtained for **F2 nanoemulsion** and **Figure 13** shows the results for **F3 nanoemulsion**. It may be noticed that the populations of approximately 300 nm appearing on juices where the nanoemulsions of the invention were added correspond to the particles in the juice and would not represent a destabilization of the original nanoemulsion, which continues maintaining its size.

Figure 14. Images of the pure orange juice (**NAR**) added with

different nanoemulsions, based on the (**F2N** & **F3N**) in a proportion of 1:40. It may be noticed that adding such a proportion does not substantially change the juice aspect.

Figure 15. Images of commercial gelatin desserts without addition (**GEL**) and, by way of example, added with the nanoemulsion, based on invention **F2** in a 1:200 proportion. Apple gelatin desserts were added such proportion (**F2 GM**), as well as orange gelatin desserts (**F2 GN**) and strawberry gelatin desserts (**F2 GF**). It may be noticed that adding such a proportion does not substantially change the gelatin dessert aspect. A portion of such gelatin (200 g) would contribute 20% of the omega-3 DRI (WHO).

DETAILED INVENTION DESCRIPTION

This invention refers, in general, to a chia oil edible nanoemulsion. In particular, it refers to a chia oil nanoemulsion comprising between 10% and 20% of chia oil (*Salvia hispánica L.*), between 2% and 5% of polysorbate, between 0.5% and 5% of at least one emulsifier other than the polysorbate, between 0.05% and 0.2% of at least one antioxidant and water. In general, the chia oil used for preparing the nanoemulsion will have between 56% and 64% of omega-3 fatty acids.

In specific preparations, the chia oil nanoemulsion, based on this invention, comprises a polysorbate selected between polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80. In other specific preparations, the chia oil nanoemulsion based on this invention comprises a mix of two or more of the polysorbates mentioned above.

In specific preparations, the chia oil nanoemulsion, based

on this invention, comprises an emulsifier selected between the phospholipids, sodium stearyl lactylate, diacetyltartaric monoglyceride, acacia gum, monoglycerides, Polyglycerin esters, propylene glycol esters, sucrose esters, and Quillaja saponins. In other specific preparations, the chia oil nanoemulsion based on this invention comprises a mix of two or more of the emulsifiers mentioned above.

In other specific preparations of this invention, the chia oil nanoemulsion comprises an antioxidant selected between ascorbic acid or its salts, polyphenols, polymethoxyflavones, and caseinomacropeptide. In other specific preparations, the chia oil nanoemulsion based on this invention comprises a mix of two or more of the antioxidants mentioned above. In very specific preparations, polyphenols are selected between fruit, tea and cocoa polyphenols and the polymethoxyflavones are citrus polymethoxyflavones.

In some specific preparations, optionally, the nanoemulsion based on this invention may also comprise at least one pH modifier. Even more specifically, the pH modifier is selected between the citric acid, the ascorbic acid and the lactic acid or a mix thereof. Optionally, the nanoemulsion based on this invention may include a mix of various of the pH modifiers mentioned herein above.

In other specific preparations, optionally, the nanoemulsion based on this invention may also comprise at least one polysaccharide. Even more specifically, such polysaccharide is selected between xanthan gum, guar gum, inulin, modified celluloses, modified starches, alginates and carragenates. Also, the nanoemulsion based on this

invention may optionally include a mix of various polysaccharides mentioned herein above.

In other specific preparations, optionally, the nanoemulsion based on this invention may also comprise at least one sugar. Even more specifically, the sugar is selected between the sucrose, glucose, fructose, maltose and lactose. Also, the nanoemulsion based on this invention may optionally include a mix of various sugars mentioned herein above.

The chia oil nanoemulsion of this invention presents a droplet size lower than 200 nm and allows the oil to be added to an aqueous medium. Also, the chia oil nanoemulsion of this invention presents a high stability to oxidation and a high bioaccessibility of omega-3 fatty acids. Thus, the chia oil nanoemulsion of this invention may be added to food so that they can contribute to the diet the required omega-3 fatty acid requirements.

The chia oil nanoemulsion of this invention presents an additional advantage and it is that it may be added to transparent drinks or food, such as jellies and desserts, without affecting their transparency and/or color. As mere examples, the nanoemulsified oil in this invention may be added to milk and dairy products (such as yogurt, cheese, etc.), infant products, seasonings, dietary supplements, desserts and jellies to improve their contribution of omega-3 fatty acids to the diet.

The chia oil nanoemulsion of this invention may be added to solid food and drinks to improve their contribution of omega-3 fatty acids to the diet without producing any fishy smell or bring cholesterol, and presenting a higher antioxidant activity.

Except as otherwise indicated, all the percentages mentioned in this document are expressed as weight of component with respect to the total formulation weight. All the documents mentioned above are attached hereto in full, as reference.

EXAMPLES

Example 1

Table 1 describes the qualitative composition of chia oil nanoemulsions, based on this invention.

Table 1

Component	% (w/w)
Chia oil	10-20
Polysorbate	2-5
Emulsifiers	0.5-5
Antioxidants	0.05-0.2
pH modifiers	0-10
Polysaccharides	0-10
Sugars	0-10
Water	Until completing 100

Example 2

Methods for obtaining oil chia nanoemulsions

Figure 1 describes a scheme of the procedure that may be used for obtaining the chia oil nanoemulsion of this invention. Such procedure may be illustrated as follows:

1- The emulsion components are weighted and the emulsifiers are dissolved in the oily or aqueous phase, based on their solubility. In some cases, the emulsifiers should be heated for dissolution.

2- A pre-emulsification is made for 3-10 min in a high-speed homogenizer.

3- The emulsification is performed by the following methods:

a- A high-pressure homogenizer between 1000 and 2000 bar.

b- In a colloid mill, between 10 and 20 min.

c- In a high-intensity ultrasound, between 10 and 20 min with cooling for dissipation of the heat generated during the process.

4- The emulsions are packed and stored at ambient temperature.

Example 3

Examples of chia oil nanoemulsions based on this invention

Table 2 describes the detailed qualitative composition of formulations **F1** to **F8**.

Table 2

	Formulations (F)							
	F1	F2	F3	F4	F5	F6	F7	F8
Chia oil (%)	10	10	10	10	10	10	20	10
Polysorbate (%)	5	5	5	5	5	4	5	3
Fractionated lecithin (%)	–	5	–	–	5	5	5	–
Skim lecithin (%)	5	–	–	–	–	–	–	–
Diacetyl tartaric monoglyceride (%)	–	–	–	2	–	–	–	–

Acacia gum (%)	-	-	1	-	-	-	-	1
Inulin	-	-	-	-	5	-	-	-
Sodium stearoyl lactylate (%)	-	-	2	-	-	1	-	2
pH regulator	0.5							
Water (%)	Up to 100							

For comparison purposes only, the formulation **F9** was also prepared containing only 10 % of chia oil, 5% of polysorbate and water.

Example 4

Droplet size distribution and macroscopic aspect of nanoemulsions

F1 to **F8** chia oil nanoemulsions were prepared with an O/W ratio of 10/90 or 20/80, where 10 or 20% (p/p) of the emulsion corresponds to chia oil. The aqueous phase was citrate buffer (pH 3). The emulsifier concentration has been expressed out of the total emulsion. The emulsifiers were solubilized in the aqueous or oily phase, depending on their solubility. A pre-emulsion was prepared in a high-speed mixer in all cases and then, such pre-emulsions were subject to ultrasound with an ultrasonic processor for 10 min, maintaining the temperature under 35°C.

The droplets sizes were determined by diluting 1:40 the nanoemulsions obtained. A dynamic light scattering (DLS) (*Zetasizer Nano-ZS, Malvern Instruments*) unit was used. The laser illuminates the sample and the light scatters at different intensities that flow at a speed that depends on

the droplet size. The data obtained were analyzed by the Contin method, from which a percentage distribution of the droplet sizes is obtained and by the method of cumulants, from which the average diameter is obtained (Martinez, M. J., Farías, E. & Pilosof, A. M. R. 2010. International Dairy Journal, 20(9), 580-588).

Figure 2 shows the droplet size distributions and the prevailing diameter value of **F1** to **F7** formulations ~~is~~ indicated in nanometers (nm). In all cases droplet sizes were obtained with prevailing diameter values between 33 and 90 nm.

Figure 3 shows images of nanoemulsions **F1** to **F3**. Nanoemulsions are noted to be translucent.

Example 5

Obtaining a nanoemulsion by different methods

Figure 4 shows the nanoemulsion droplet size distributions, based on invention **F8**, obtained by high-intensity ultrasound or in a high-pressure homogenizer. It is noticed that both methods may produce a nanoemulsion, whose main population has a droplet diameter of 79 nm.

Example 6

Physical stability

The stability of emulsions was determined at 25°C, by the droplet size average diameter along the time. **Figure 5**, shows the results obtained for **F1** & **F4** formulations. In both cases, it was noticed that the emulsions kept stable for two months.

Example 7Oxidative stability

The peroxide index (PI) is a measure of the oxygen bound to fats as a peroxide, i.e., representing the determinable amount of active oxygen contained in 1 kg of sample and is expressed as milliequivalents/kg of oil (meq/kg of oil). As primary oxidation products hydroperoxides are specially formed, in addition to reduced amounts of other peroxides. The PI provides information about the degree of oxidation of a sample and allows estimating the degree of alteration of the fat. However, if the oxidation is very advanced, the peroxides start degrading to other products of the oxidative process, which decreases the PI. The test was performed by storing the samples in the dark at 37°C for seven days (based on the method in *Shantha N. C. & Decker E. A. 1994. Journal of AOAC International vol 77, no. 2 421-424*). **Figure 6** shows the PI evolution of the original chia oil, of **F2** nanoemulsion and the last with added natural antioxidants, based on **Table 3**.

Table 3

	Formulations (F)		
	F10	F11	F12
Chia oil (%)	10		
Polysorbate (%)	5	5	5
Fractioned lecithin (%)	5	5	5
Ascorbic acid (%)	0.1	–	0.05

Green tea polyphenoles (%)	-	0.1	0.05
pH regulator	0.5		
Water (%)	Up to 100		

The chia oil has initially a PI of 1.55 meq/kg oil, a value that may be compared to the values reported in other sources (see, for example, the application PCT **WO2008041834**). Then, such value increases with the oil self-oxidation, reaching 3.38 meq/kg. The **F2** nanoemulsion presented a degree of oxidation higher than the oil, reaching 5.5 meq/kg. This is due to the higher interfacial area of the nanoemulsified oil (droplets). The synergistic combination of both natural antioxidants (**F12**) results in PI values that are maintained in all cases below the chia oil values and is appropriate for stabilizing the nanoemulsions against the oxidation. The values were much lower than the PI limit established by the *Codex Alimentarius* (*Codex Alimentarius Commission*. Codex Stan 19. Edible fats and oils not covered by individual standards) for edible oils (10 meq/kg of oil).

Example 8

Bioaccessibility of fatty acids of nanoemulsified chia oil

The bioaccessibility of omega-3 fatty acids was evaluated by determining the fatty acids released (% FFA) of the chia oil and its nanoemulsions during an *in vitro* gastroduodenal digestion process. It consisted in a gastric stage (1 hour, pH 2.5, 37°C), followed by a duodenal stage (1.5 hour, pH 7, 37°C), with the presence of enzymes, biliary salts and other characteristic components of the medium, at

physiological concentration, based on the protocol used by Bellesi & col., (2016, Food Hydrocolloids, 52, 47-56). The volume of NaOH required for maintaining the pH at 7 during the duodenal stage (automatically dosed by means of a *pH stat titration*), is related to % FFA based on the following equation:

$$\% \text{ FFA } = ((V_{\text{NaOH}(t)} * M_{\text{NaOH}} * M_{\text{TG}}) / m_{\text{TG}} * 2) * 100$$

where M_{NaOH} is the molar concentration of the NaOH solution used, M_{TG} is the average molecular weight of TG that is present in the chia oil and m_{TG} is the TG mass existing in the reaction mixture when the pancreatic lipase is added.

The nanoemulsion bioaccessibility was evaluated, based on the **F1** invention during the *in vitro* duodenal digestion. For comparative purposes, a nanoemulsion is shown containing a single polysorbate emulsifier at 5% (**F9**).

Figure 7 shows the release profile of fatty acids during the *in vitro* duodenal digestion. A higher rate and extent of lipolysis (greater than 60%) is observed for nanoemulsions as compared to non-emulsified chia oil.

The composition of emulsifiers influences the FA bioaccessibility. Thus, the **F1** nanoemulsion presented a higher release of fatty acids (80%) in comparison with the nanoemulsion **F9**. That is to say, the presence of other emulsifiers in combination with the polysorbate increases the FA bioaccessibility.

Example 9

Applications of the invention nanoemulsions to final products

The nanoemulsions must be compatible and stable in the end product. This implies that they should not alter the appearance, taste, texture, etc., of the product in which they will be added. Fortifying transparent or slightly opaque products such as beverages (juices, flavored waters, sports drinks, etc.) or gelatins represents a technological challenge, since a nanoemulsion should be achieved that does not only have a very small droplet size (total transparency is achieved for droplet sizes less than 50 nm), but also to remain stable in the final product.

It is easier to fortify non-transparent products, such as milk, yogurts, dairy products, as a not so small droplet size is required. However, the small size is also convenient for the achievement of a high stability.

The application of some nanoemulsions in apple juice (crystalline appearance) and in orange juice (turbid appearance) is shown below. In the case of the orange juice, the solids in the suspension have been removed to be able to characterize the stability of the nanoemulsion added to the juice.

1) Commercial apple juice

a) Distribution of the droplet size and stability of the apple juice in which chia oil nanoemulsions were added

The distribution of the chia oil droplet size in the nanoemulsion is shown along the time, as well as in apple juice where the invention nanoemulsion in a 1:40 proportion has been added. The prevailing diameter value in nanometers (nm) has been indicated. For comparative purposes, the distribution of the pure apple juice particle size is

shown. **Figure 8** shows the results obtained for the apple juice in which a nanoemulsion has been added, based on invention **F2** and **Figure 9** shows the results for apple juice in which a nanoemulsion has been added based on invention **F3**. It may be noticed that the populations of approximately 300 nm appearing on juices where the nanoemulsions of the invention were added correspond to particles in the juice and would not represent a destabilization of the original nanoemulsion, which continues maintaining its size (40 - 60 nm).

b) Turbidity and macroscopic aspect of apple juice in which chia oil nanoemulsions have been added

The turbidity of the apple juice in which different nanoemulsions were added was measured with a spectrophotometer at a wave length of 600 nm. The nanoemulsions based on invention **F1** & **F3** were added to the apple juice in a 1:40 & 1:200 proportion, the first one representing the daily recommended intake (DRI) of omega-3 (World Health Organization, WHO) in a portion (200 ml) and the second one 20 % of the DRI by portion. Also, a pure apple juice was measured as control (**Figure 10**). It may be noticed that such apple juices in which the invention emulsions were added in a 1:40 proportion presented a turbidity 4 - 5 times higher than the pure juice. Instead, when they are added in a 1:200 proportion, the apple juice presents a very low turbidity, as compared with the pure juice. **Figure 11** shows images of pure apple juice (**MAN**) added with different nanoemulsions, based on the invention, in 1:40 & 1:200 proportions.

Therefore, it may be concluded that nanoemulsions based on this invention under evaluation (**F1** & **F3**) may be added to

crystalline apple juice in a 1:200 proportion, without substantially changing their aspect and maintaining their integrity (stability along the time) and flavor. In commercial apple juices with a higher degree of opacity, the invention nanoemulsions may be added in a higher proportion without altering their appearance.

2) Commercial orange juice

Distribution of the droplet size and stability of the orange juice in which chia oil nanoemulsions were added

Figures 12 & 13 show the distribution of the chia oil droplet size along the time in the original nanoemulsion, as well as in apple juice where a nanoemulsion, based on the invention, in a 1:40 proportion has been added. The prevailing diameter value in nanometers (nm) has been indicated. For comparative purposes, the distribution of the pure orange juice particle size is shown. **Figure 12** shows the results obtained for the orange juice in which a nanoemulsion has been added, based on invention **F2** and **Figure 13** shows the results for orange juice in which a nanoemulsion has been added based on invention **F3**.

It may be noticed that the populations of approximately 300 nm appearing on juices where the nanoemulsions of the invention were added correspond to the particles in the juice and would not represent a destabilization of the original nanoemulsion, which continues maintaining its size (33 - 59 nm).

Figure 14 shows images of pure orange juice (**NAR**) added with different nanoemulsions, based on the invention, in a 1:40 proportion.

It may be concluded that nanoemulsions based on this invention under evaluation (**F2 & F3**) may be added to a commercial orange juice in a 1:40 proportion, without substantially changing its aspect and maintaining its integrity (stability along the time) and flavor.

3) Commercial Gelatin Dessert

Figure 15 shows images of commercial gelatin desserts without additions (**GEL**) and, for example, added with a nanoemulsion, based on invention **F2** in a 1:200 proportion. Different flavors were evaluated: apple (**GEL M**), orange (**GEL N**) and strawberry (**GEL F**) for evaluating the influence of the nanoemulsion in the general aspect and flavor of such desserts.

It is concluded that the nanoemulsion based on the invention under evaluation (**F2**) may be added to a commercial gelatin dessert in a 1:200 proportion without substantially changing its aspect. No differences were identified in their flavor and consistency.

CLAIMS

1. An edible chia oil nanoemulsion, comprising:
between 10% and 20% of chia oil (*Salvia hispanica L.*),
between 2% and 5% of polysorbate,
between 0.5% and 5% of at least one emulsifier other than polysorbate,
between 0.05% and 0.2% of at least one antioxidant, and
water.
2. A nanoemulsion according to claim 1, wherein the polysorbate is selected between polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80 or a mix thereof.
3. A nanoemulsion according to claim 1, wherein the emulsifier is selected between the phospholipids, sodium stearyl lactylate, Diacetyl Tartaric Acid Ester of Mono- and Diglycerides (DATEM), acacia gum, monoglycerides, Polyglycerin esters, propylene glycol esters, sucrose esters, and Quillaja saponins or a mix thereof.
4. A nanoemulsion according to claim 1, wherein the antioxidant is selected between ascorbic acid or its salts, polyphenols, polymethoxyflavones, caseinomacropeptide or a mix thereof.
5. A nanoemulsion according to the previous claim, wherein the polyphenols are selected between fruit, tea and cocoa polyphenols and because the polymethoxyflavones are citrus polymethoxyflavones.
6. A nanoemulsion according to any of the previous claims, comprising at least one pH modifier.

7. A nanoemulsion according to any of the previous claims, wherein the pH modifier is selected between the citric acid, the ascorbic acid and the lactic acid or a mix thereof.

8. A nanoemulsion according to any of the previous claims, comprising at least one polysaccharide.

9. A nanoemulsion according to any of the previous claim, wherein the polysaccharide is selected between xhantan gum, guar gum, inulin, modified celluloses, modified starches, alginates and carragenates or a mix thereof.

10. A nanoemulsion according to any of the previous claims, comprising also at least one sugar.

11. A nanoemulsion according to any the previous claims, wherein the sugar is selected between the sucrose, glucose, fructose, maltose and lactose or a mix thereof.

12. An edible formulation that contains omega-3 fatty acids comprising a chia oil nanoemulsion based on any of claims 1 to 11.

13. An edible formulation according to the previous claim, wherein it is in a liquid, solid or gel form.

14. An edible formulation according to the previous claim, wherein it is a solid formulation, a liquid formulation, a dairy product, an infant edible product, a drink, a seasoning, a dietary supplement, a dessert or a jelly.

15. An edible formulation according to the previous claim, wherein it is a liquid formulation.

16. An edible formulation according to the previous claim, wherein it is a transparent liquid formulation.

17. An edible formulation according to claim 14, wherein it is a jelly.

18. An edible formulation according to the previous claim, wherein it is a transparent jelly.

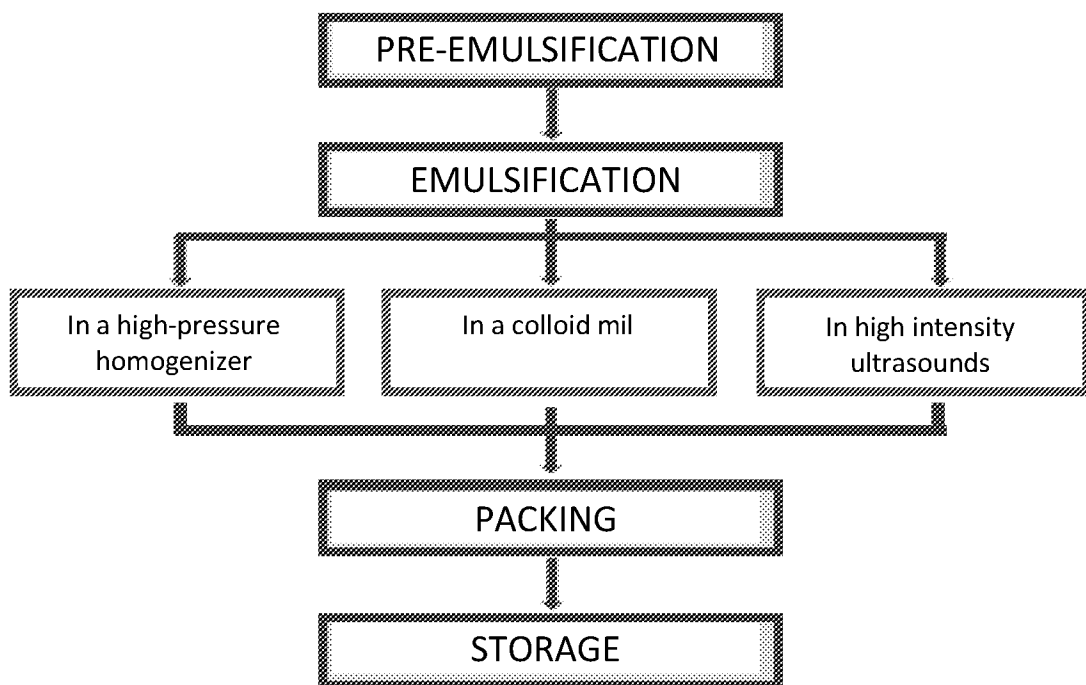


FIGURE 1

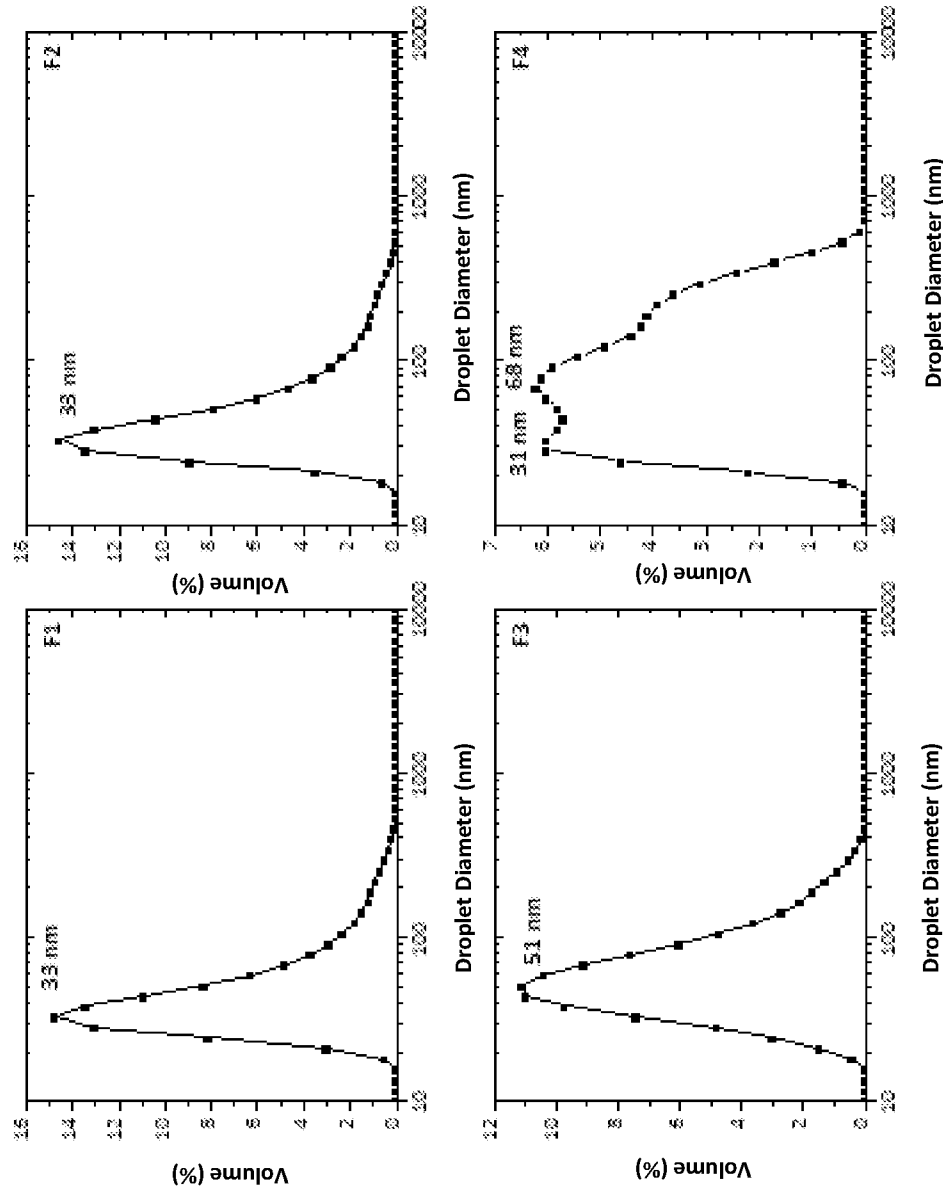


FIGURE 2

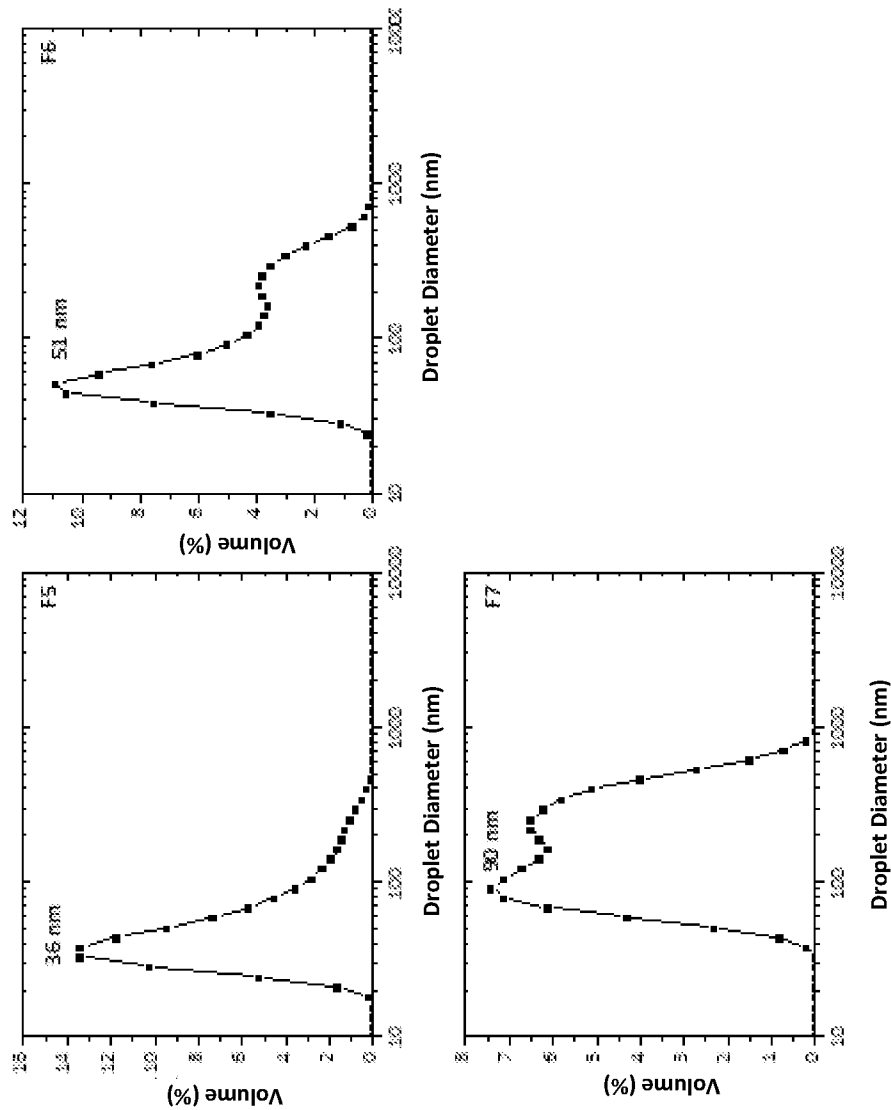


FIGURE 2 (CONT.)

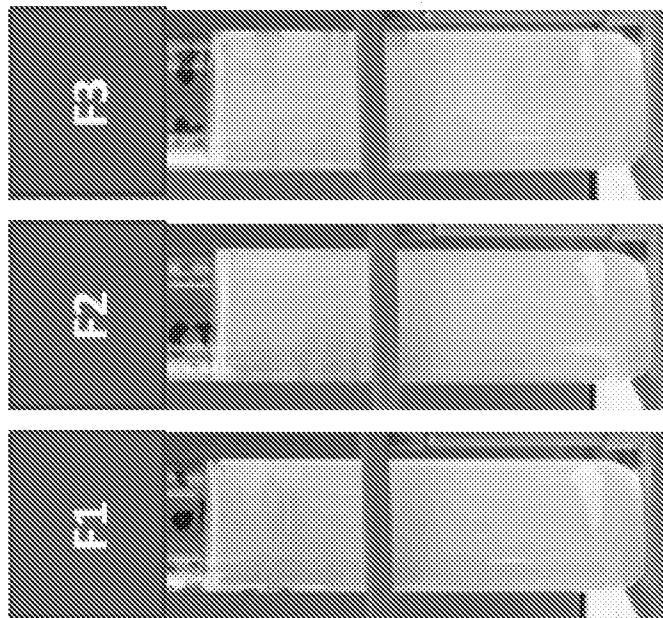


FIGURE 3

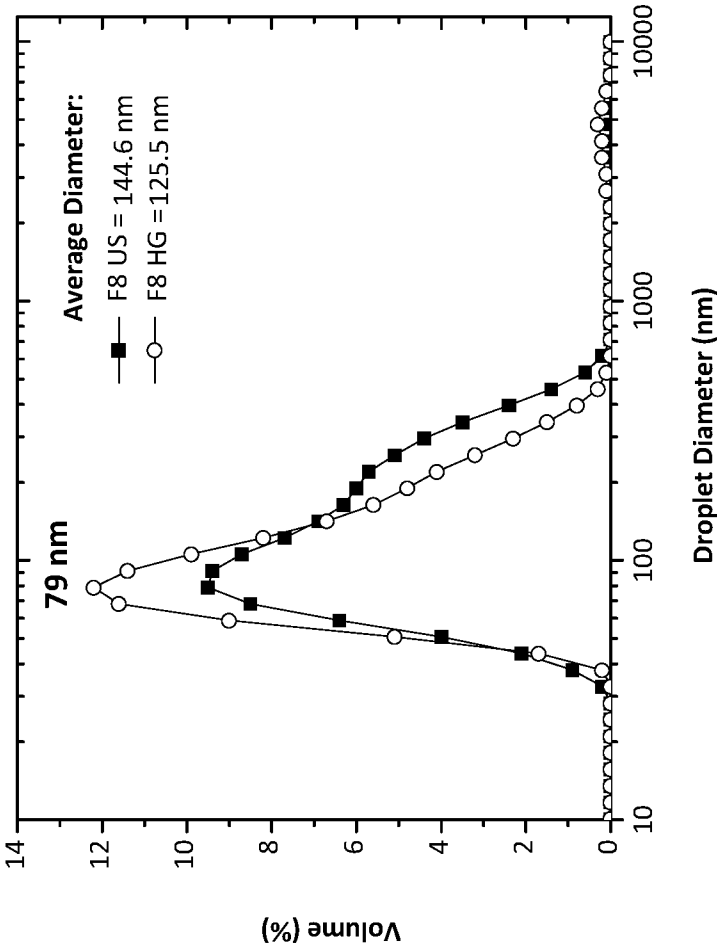


FIGURE 4

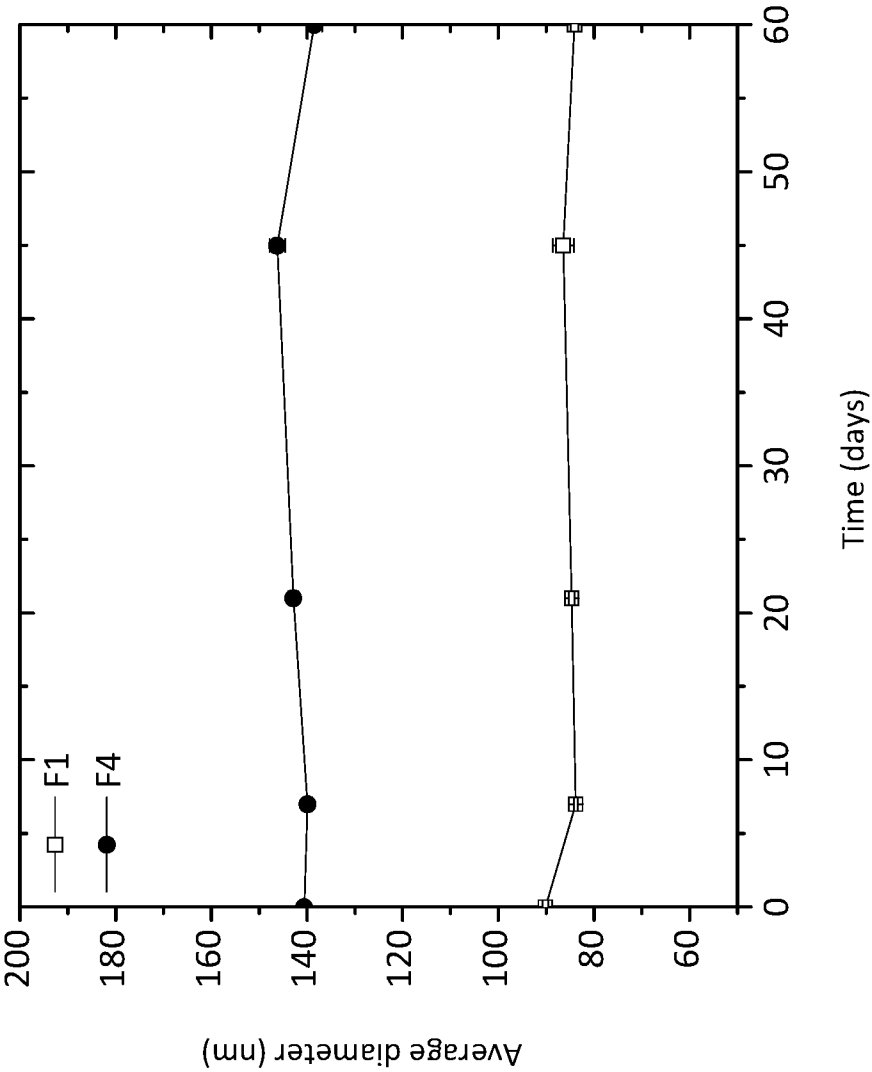


FIGURE 5

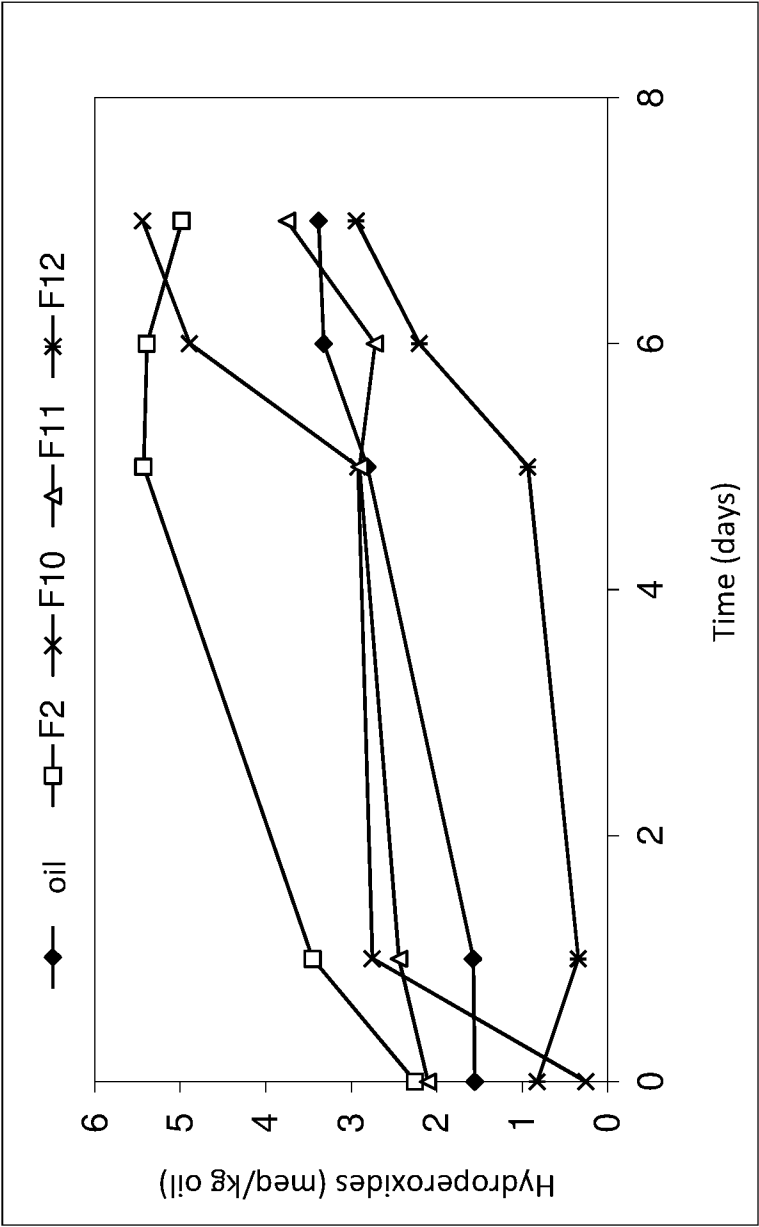


FIGURE 6

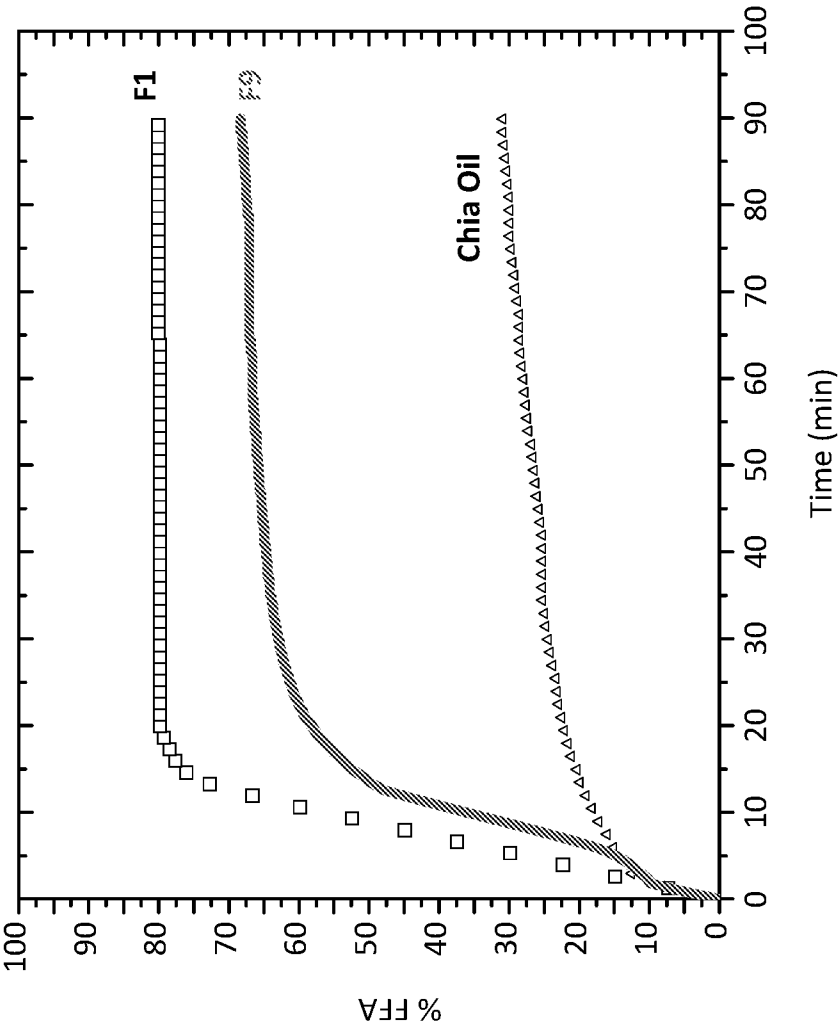


FIGURE 7

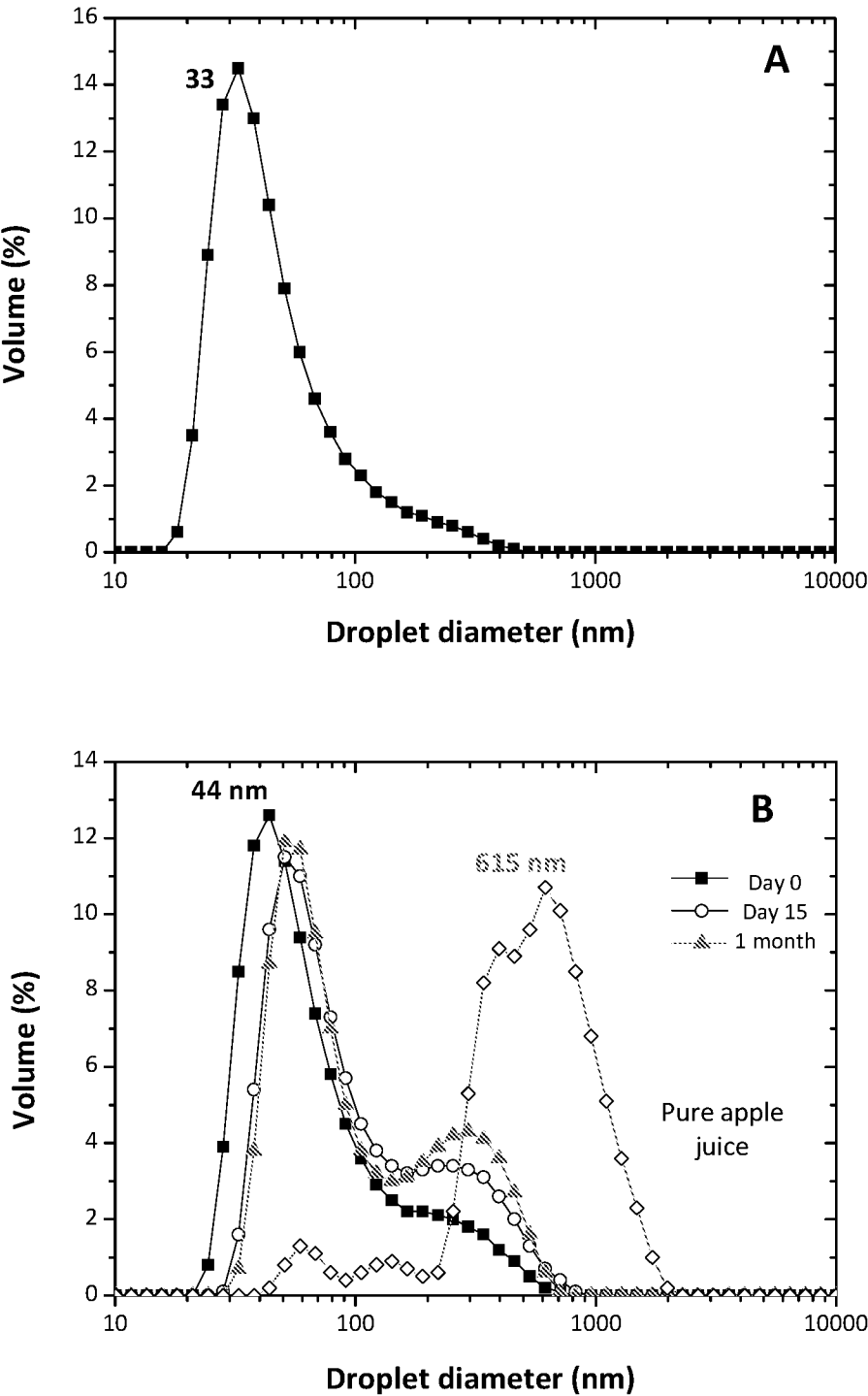


FIGURE 8

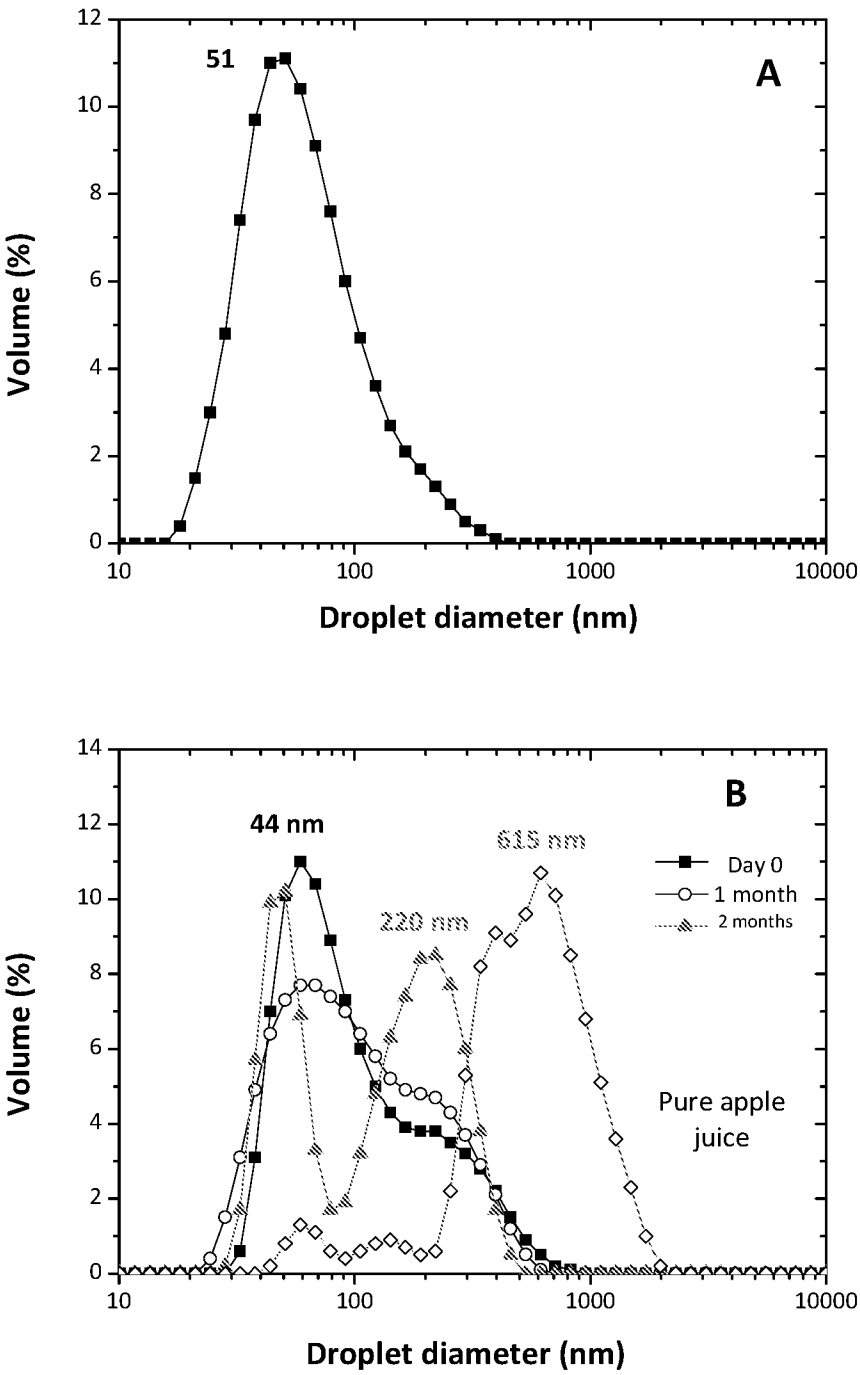


FIGURE 9

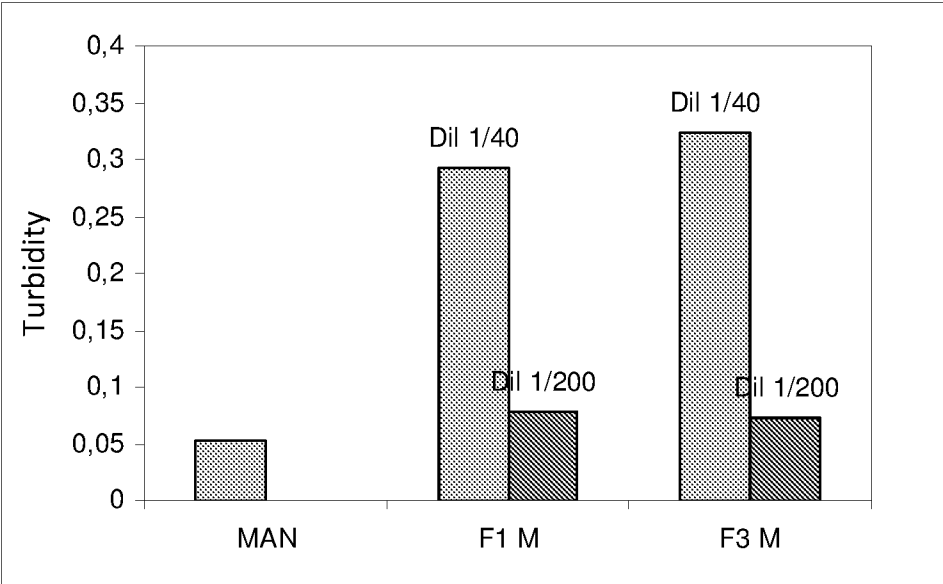


FIGURE 10

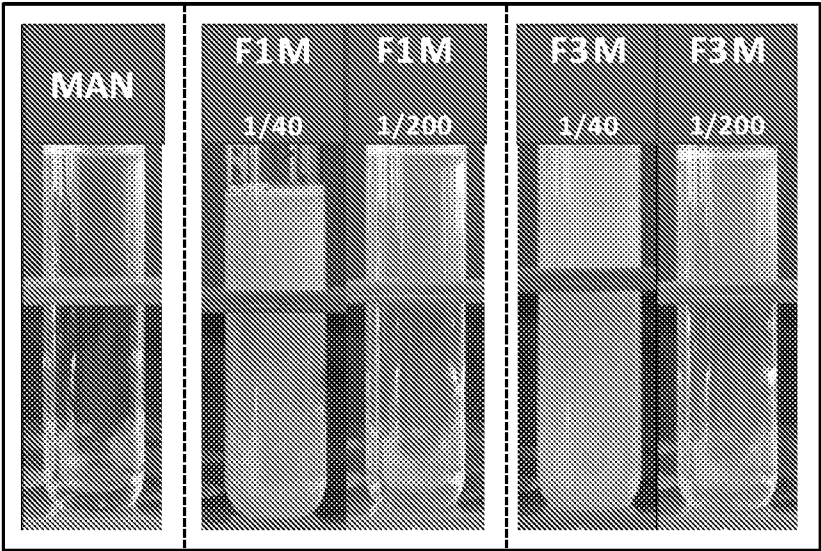


FIGURE 11

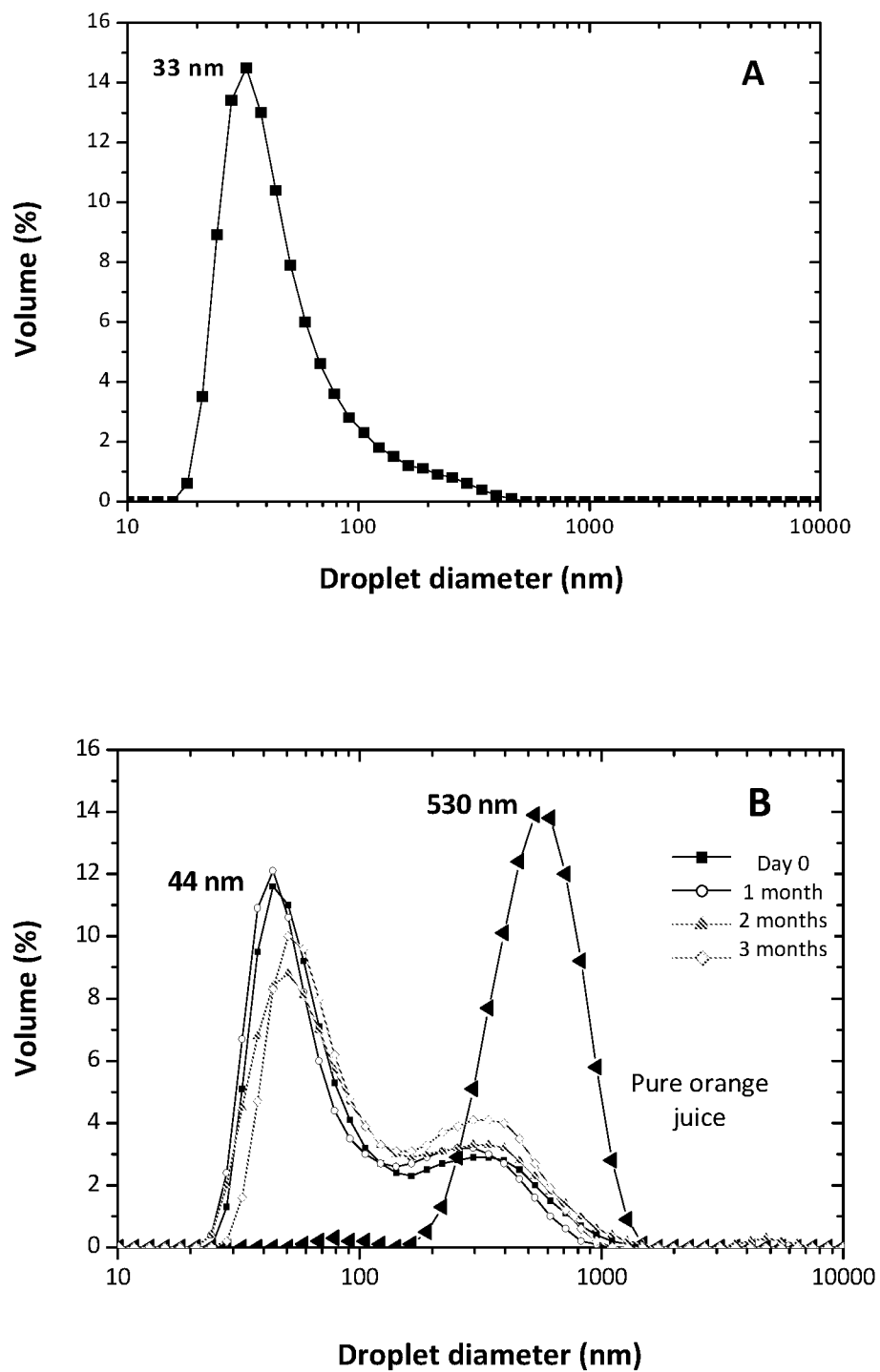


FIGURE 12

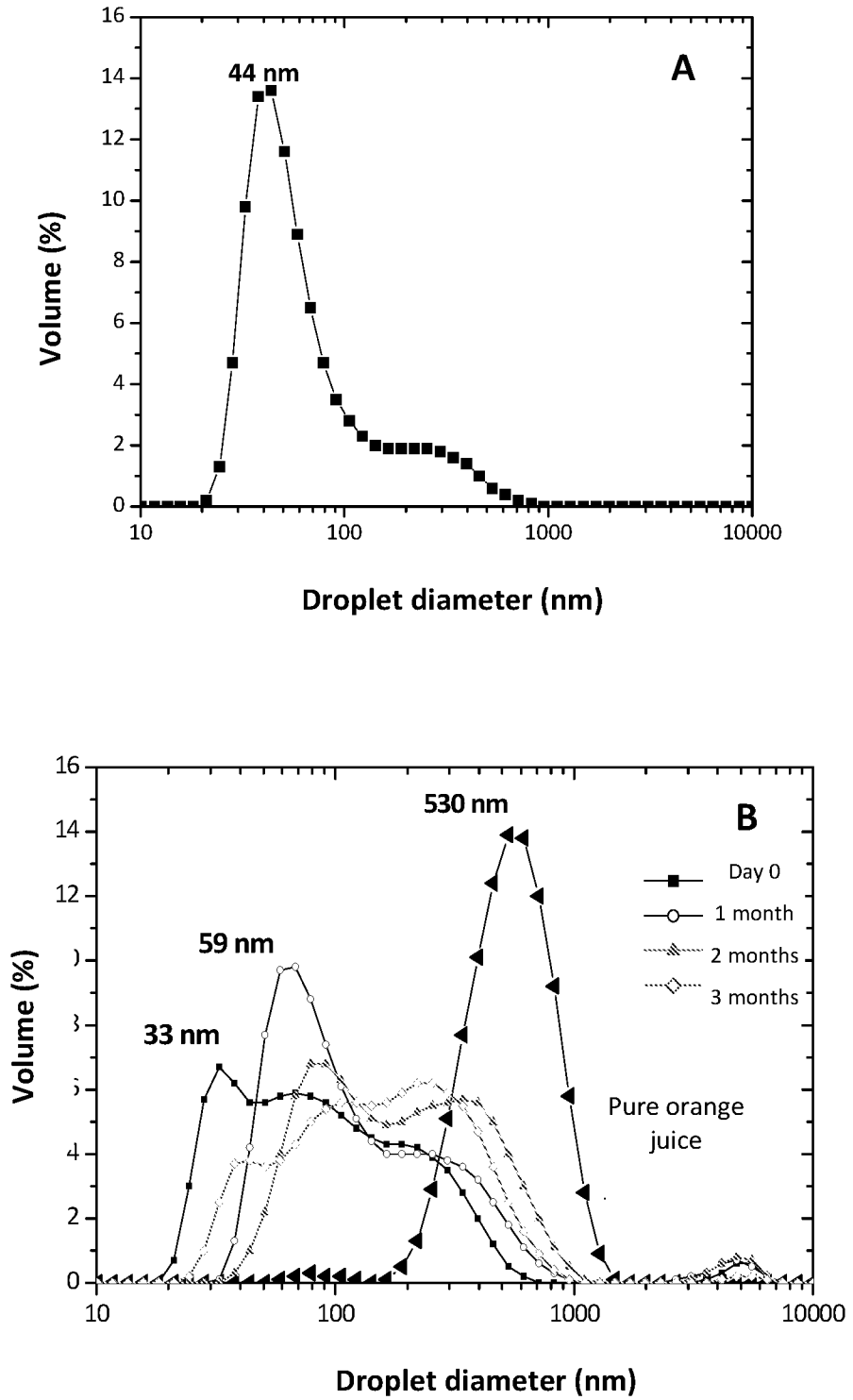


FIGURE 13

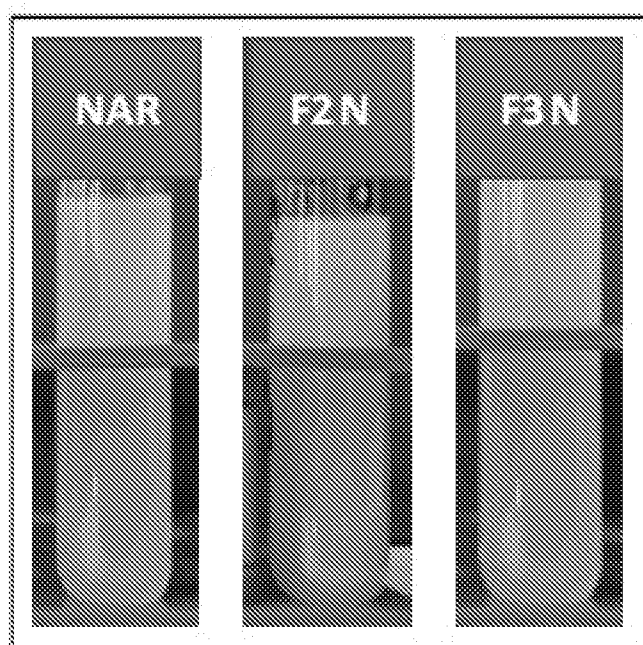


FIGURE 14

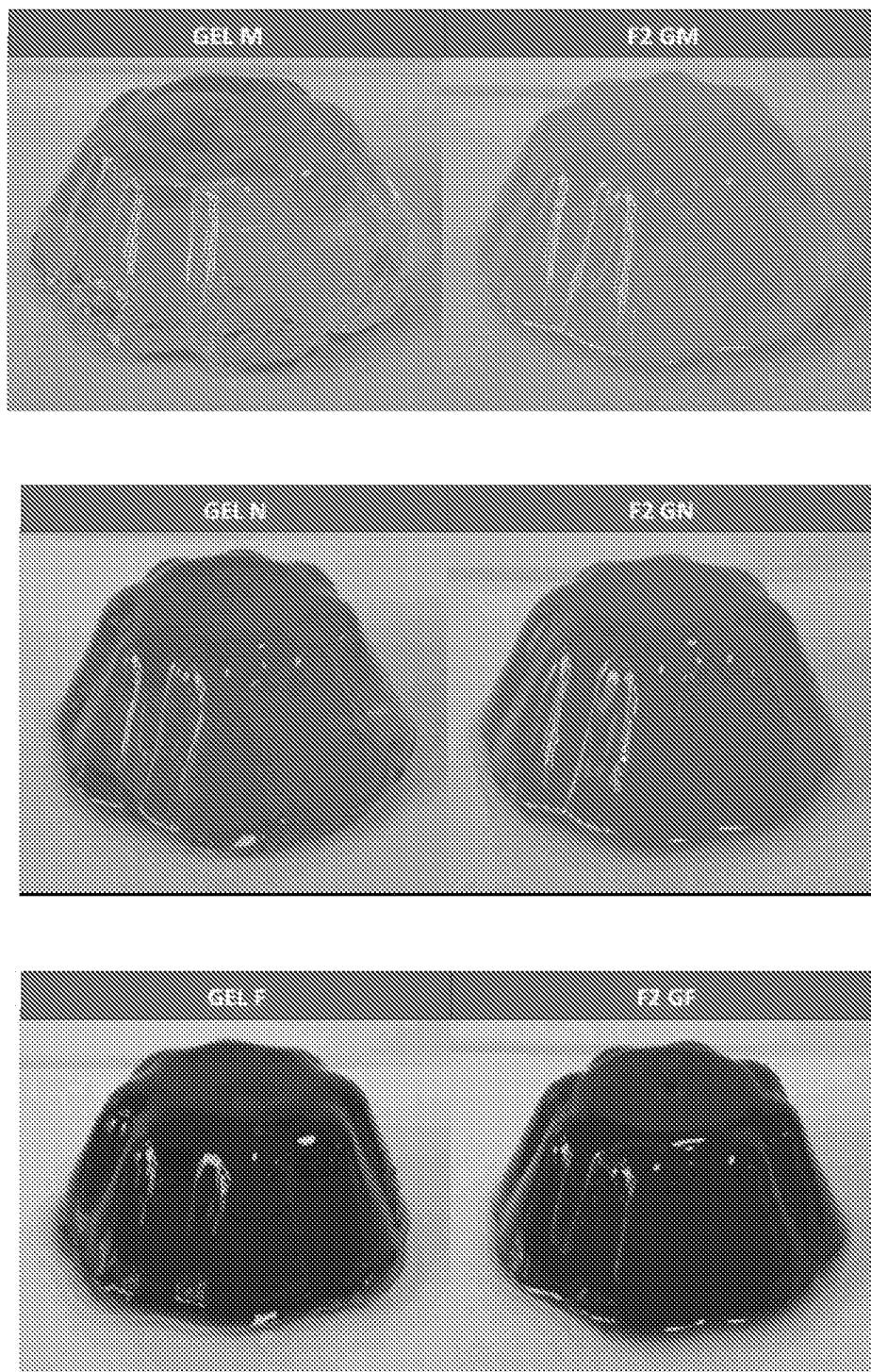


FIGURE 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/054880

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L33/115 A23L29/10 A23L29/30 A23L33/105 A23L2/52
A23D7/00 A23D7/005 A61K9/107 A61K47/06 A61K47/44
A61K47/14

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)

A23L A23D A61K

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, BIOSIS, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/047259 A1 (GLAXOSMITHKLINE LLC [US]; ABU-BAKER OMAR ABDELFAH [US]; MACKENZIE D) 21 April 2011 (2011-04-21)	1,2,4,5, 12-18
Y	Formulation 7; page 29 - page 30; claims 1-40; table 2 page 4, line 27 - page 8, line 6 page 9, line 13 - page 11, line 30 -----	3,6-11
Y	US 2010/298435 A1 (ABRIL JESUS RUBEN [US] ET AL) 25 November 2010 (2010-11-25) paragraph [0007] - paragraph [0017]; example 2 paragraph [0025] - paragraph [0041] -----	3,6-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 November 2017

Date of mailing of the international search report

16/11/2017

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Authorized officer

Munteanu, I

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2017/054880

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