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Physicochemical and Microstructural Stability of Food-Grade Olive Oil Nanoemulsions Prepared by Low-Energy Emulsification

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Olive oil attributes to innumerable health benefits due to presence of minor constituents including triterpenic acids and phenolics, as well as monounsaturated fatty acids (MUFA). Its utilization is limited due to poor aqueous solubility hence it requires an appropriate colloidal delivery system. The formation of oil-in-water nanoemulsion may improve its solubility by producing small size nano droplets with the help emulsion phase inversion approach (low energy emulsification technique). Electrical conductivity and rheological study confirmed phase inversion in the nanoemulsion preparation. A total of 18 nanoemulsion samples were prepared with olive oil at different concentrations (0.5, 1, and 2%), surfactant-oil-ratio (SOR) (1, 2) and mixing speed (500, 1000 and 1500 rpm). All the nanoemulsions were stable to phase separation. Formulation viz. olive oil (2%), SOR 2 and mixing speed of 1500 rpm were obtained as the best optimized condition to produce smallest droplet size and polydispersity index (PDI). The nanoemulsion developed with the optimized formulation was subjected to environmental stress conditions commonly used in food processing operations (heat treatment: 65, 95, 121°C; pH: 1, 3, 7; NaCl concentration: 0.1, 0.5, 1M). Olive oil nanoemulsion was found stable to in these stress environments. Transmission electron microscope (TEM) study observed less increase in the droplet growth during one month storage of nanoemulsion at 4°C indicating better stability of the emulsion during storage.

Keywords: Droplet size, Emulsion phase inversion, Mixing speed, Polydispersity, Storage stability

Introduction

Olive oil contains various nutritional constituents namely oleic acid (MUFA), squalene and various phenolic compounds i.e. tyrosol, hydroxytyrosol, tocopherols and oleuropein.[1,2] Owing to the presence of these bioactive compounds, olive oil exerts several health benefits such as antioxidant, anti-ageing, anti-atherosclerotic, anti-platelet aggregation and anti-inflammatory effects.[3] However, the lipophilic compounds present in olive oil are generally hydrophobic in nature and couldn't easily miscible with aqueous phase thus require an appropriate delivery system to incorporate into pharmaceutical and food products. Nanoemulsions formulation is one of the promising approaches by which bioactive components can be delivered through immiscible phases into functional foods/ingredients. Nanoemulsions, due to their small droplet size, acquire good kinetic stability thereby augments bioavailability.

Oil-in-water nanoemulsion consists of dispersion of oil phase into aqueous phase. Emulsifiers play an important role in providing sufficient interfacial tension to emulsions by providing steric or electrostatic repulsion. Nanoemulsions have droplet size less than 200 nm thus offer enormous advantages over conventional emulsions. Nanoemulsions have optical clarity because of their small droplet size, which is a crucial reason for their relevance in food matrices.[4] Moreover, physical stability of nanoemulsion can be improved by reducing phase separation, coalescence, and flocculation.[5] In general, there are two approaches i.e. high-energy or low-energy are used for preparation of nanoemulsions. High-energy techniques need the utilization of complex equipment including sonicator, microfluidizers, and high-pressure homogenizers.[6] Low-energy techniques, on the other hand, exploit surfactant characteristics to form nanoemulsions. Spontaneous emulsification, emulsion phase inversion, phase inversion temperature, and phase inversion composition techniques are examples of low-energy techniques.[7] These approaches are simpler to implement due to low equipment cost as well as their ability to

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produce very fine droplets than high-energy techniques.[8] The low-energy approach involves negligible heat generation with respect to high-energy approach that helps to prevent the degradation of heat-sensitive materials. Despite various advantages, the low-energy approach has not been explored in food applications due to inadequate knowledge of its preparation mechanisms.[9]

Earlier studies conducted on the development of olive oil nanoemulsions by emulsion phase inversion and effect of different processing variables on its stability is scanty. Previous studies related to emulsion phase inversion have revealed that the emulsion droplets size depends upon various processing conditions including the category and concentration of surfactants and oil phase, surfactant-oil ratio and stirring conditions. Therefore, the purpose of the current investigation was to scrutinize the consequence of processing variables on the formation and physical stability of olive oil nano emulsions. Further, rheological, environmental stress stability and microstructure stability was also investigated. The results obtained from this study will offer a significant contribution to the field of low-energy nanoemulsion preparation and their use in food formulations.

Materials and Methods

Olive oil and Tween 80 were procured from Sigma Aldrich Co. (St. Lois, MO, USA). All other reagents used in the study were of analytical grade.

Nanoemulsions Preparation

Olive oil and Tween 80 were properly mixed in a beaker. Aqueous phase (Milli-Q water) was added gradually to this mixture with continuous mixing. Mixing process continued for 4 h to make sure the formation of oil-in-water nanoemulsion droplets. The 18 different emulsion combinations as shown in Table 1 are prepared from olive oil (0.5–2% w/w), surfactant-to-oil-ratio (SOR) (1 & 2) and mixing speed (500, 1000 and 1500 rpm). As SOR varied from 1 to 2, consequently Tween 80 concentration (0.5 - 4% w/w) and aqueous phase (94 – 99% w/w) also varied to keep the total emulsion capacity as 100 g.

Droplet size, Zeta Potential and Polydispersity Index (PDI)

Droplet size was measured using Dynamic light scattering (DLS) method conducted at 25 ± 1°C temperature and scattering angle of 90° (Zetasizer Nano-ZS 90, Malvern Instruments, UK). The samples were put into square 4-clear wall cuvettes of side length 10 mm and outer diameter 12 mm. The refractive indices of dispersed phase i.e. olive oil and dispersion medium i.e. aqueous phase were taken as 1.53 and 1.33. Samples were diluted 100 times to avoid multiple scattering in the analysis process. Every individual measurement was derived from an average of thirteen runs. Zeta potential was measured using disposal cuvettes and each individual measured were the average of thirteen scans.

Stability under Different Environmental Conditions

Different conditions of heat treatment, pH, and ionic strength were applied to nanoemulsions to test their stability.

Thermal Stability

The heat shock stability of nanoemulsion was measured by applying thermal treatment for certain

Table 1 — Process formulations prepared to optimize the Tween 80 based nanoemulsions

Mixing speed (rpm)	Olive oil concentration (% wt)	Tween 80 concentration (% wt)	SOR	Aqueous phase concentration (% wt)
500	0.5	0.5	1	99
		1	2	98.5
	1	1	1	98
		2	2	97
	2	2	1	96
		4	2	94
1000	0.5	0.5	1	99
		1	2	98.5
	1	1	1	98
		2	2	97
	2	2	1	96
		4	2	94
1500	0.5	0.5	1	99
		1	2	98.5
	1	1	1	98
		2	2	97
	2	2	1	96
		4	2	94

time duration. 10 mL of samples were taken in a test tube of 20 mL capacity and heated in a water bath at 65°C/30 min and 95°C/15 min.

pH Stability

Twenty mL of nanoemulsion was taken into glass beakers of 50 mL volume followed by adjusting the pH of samples to 1, 3 and 7 using NaOH/HCl of sufficient strength (0.1 M) and (1M) respectively.

Ionic Strength

The ionic strength was examined by adding different NaCl salt solution of 0.1, 0.5 and 1M into nanoemulsion samples. The particle size and zeta potential of nanoemulsions were analyzed after all above environmental stress conditions employed.

Transmission Electron Microscopy(TEM)

A drop of the olive oil-in-water nanoemulsion was taken for microstructure study by positioning it on a 200-mesh carbon coated copper grid. Phosphotungstic acid (2%) was used for negatively staining nanoemulsion sample. The grid was left to air dry for 30 min before analyzing the image captured by TEM. The grid was imaged using Transmission electron microscopy (FEI Tecnai G2-20S, Netherlands) operating at 200 kV. Images were recorded with help of Gatan US1000 (2k × 2K CCD camera) (Gatan Inc., US).

Electrical Conductivity

The electrical conductivity of nanoemulsions was evaluated to confirm the occurrence of phase inversion at different levels (%) of water addition using electrical conductivity meter (Accumet AP85; Fisher Scientific, Pittsburgh, PA).

Rheological Analysis

The rheological characterization of nanoemulsion was done by using mechanical bearing rheometer (MCR 52; M/s. Anton Paar, GmbH, Austria) provided with RHEOPLUS/32 software. The cone-and-plate geometry probe (CP 75/1°, 75 mm diameter, 1° cone angle) was used to carry out the oscillatory rheology to determine storage modulus (G') and loss modulus (G") of nanoemulsions.

Statistical Analysis

All measurements were expressed as mean ± standard error obtained from the average of triplicates of each individual result. Statistical analysis was carried out by ANOVA and significant difference among means ($p<0.05$) were obtained with Duncan post-hoc test using SPSS software (IBM, USA).

Results and Discussion

Effect of Mixing Speed and SOR on Droplet Size of Nanoemulsion

The droplet size of olive oil-in-water nanoemulsions prepared by means of Tween 80 at different SOR and mixing speeds are presented in Table 2. It is clear from the obtained results that the droplet size of nanoemulsions were influenced by all the process variables employed. It was found that the droplet size of nanoemulsions reduced with increase in mixing speed from 500 to 1500 rpm with a few exceptions. The higher stirring speeds resulted in reduced droplet sizes when the emulsifier concentrations were kept low, where as higher emulsifier concentrations and SOR values dominated the stirring speed and produced even smaller droplet sizes.[10] In fact, at high SOR values the bi-continuous microemulsion (oil/water/oil) formed at upper boundary during intermediate stage dispersed rapidly into the aqueous phase without extensive stirring.[11] It is evident from the Table 2 that droplet size of nanoemulsions ranges between 131.47 ± 2.13 to 639.53 ± 26.21 nm. The smallest droplet size (131.47 ± 2.13 nm) was obtained at high SOR value 2 when Tween 80 was employed for emulsion preparation at a mixing speed of 1500 rpm and at an oil concentration of 2%. The droplet size of vitamin E acetate nanoemulsions produced using Tween 80 was decreased as the stirring speed increased from 200 to 800 rpm.[9] Few studies revealed that stirring speed had no influence on droplet size in the low-energy nanoemulsion processing as the chemical energy in the system being the key driving force for helping self-

Table 2 — Droplet size (nm) of olive oil nanoemulsions prepared using Tween 80

Mixing speed (rpm)	Olive oil(%w/w): SOR					
	0.5:1	0.5:2	1:1	1:2	2:1	2:2
500	205.13^{aC} ± 11.13	251.43^{dC} ± 11.33	228.07^{bA} ± 15.52	247.27^{cC} ± 16.01	639.53^{fC} ± 26.21	329.67^{eC} ± 36.28
1000	192.2^{bB} ± 29.12	232.13^{dB} ± 20.31	353.60^{eC} ± 44.62	215.93^{cB} ± 11.83	496.43^{fB} ± 67.6	153.10^{aB} ± 2.13
1500	187.40^{cA} ± 2.26	213.13^{dA} ± 15.63	287.60^{eB} ± 2.42	157.73^{bA} ± 4.08	426.83^{fA} ± 17.55	131.47^{aA} ± 2.13

Mean ± Standard error (n=3); Significant differences in mean values ($p<0.05$) are indicated by different superscripts in the row (a,b,c,d,e,f) and column (A,B,C) assembly of surfactant molecules.[12] In fact, every system shall be investigated separately as the components of emulsion matrix and their concentrations affect the emulsion stability mechanisms.[13] The authors advocated that mixing is required to facilitate the surfactant movement to oil-water interphase and to promote the disorder of bicontinuous microemulsion occurred at the boundary of the oil and aqueous phase to obtain smaller droplets.

Olive oil (0.5%) at SOR 2 produced bigger droplet size than SOR 1, since Tween 80 used for emulsification was only 0.5–1% thus insufficient to prevent droplet aggregation. There may be inadequate emulsifier available to coat the oil droplet surfaces in the nanoemulsion at low SOR values thus led to droplet coalesce resulting in bigger droplet size.[14] As the oil concentration increased further (1 & 2%), SOR 2 resulted in significantly smaller droplet sizes than SOR 1 ($p<0.05$). As the olive oil concentration was enhanced from 1–2% subsequently emulsifier concentration in the matrix was also enhanced from 1–4% thus might have effectively inhibited droplet aggregation. The increase in the droplet size with increase in SOR values could be due to the formation of liquid crystals of emulsifier that are difficult to disrupt during mixing after critical limit of SOR achieved.[13]

Effect of Mixing Speed and SOR on the Polydispersity Index (PDI) of Nanoemulsion

PDI values of prepared nanoemulsions are presented in Table 3. PDI indicates the heterogeneity of emulsion droplet size. According to international standards organization (ISO) emulsions with PDI value of <0.05 are monodisperse and PDI value of >0.7 are polydisperse.[15] Smaller PDI value of 0.41 ± 0.02 was obtained for nanoemulsion prepared using 1% oil phase, 2% oil at SOR 2. The PDI value decreased with increase in SOR at all oil phase concentrations. Our results are in line with the prior studies who revealed that poly dispersed emulsion droplets obtained at low SOR's (0.05–0.25) and mono dispersed emulsion droplets being found at higher SOR's (0.5-2).[16] Due to small poly dispersity index at higher mixing speed, the droplet size distribution was further studied at 1500 rpm (Fig. 1). It was observed that nanoemulsions formed were poly disperse with broad droplet size distributions. In case of nanoemulsion prepared using 0.5% oil and SOR1 obtained a sharp peak with mono-modal like distribution while its droplet size was larger when compared to nanoemulsion prepared using 2% oil and SOR 2. The greater variation in the droplet size distribution was observed while adding oil phase drop wise during emulsion preparation using catastrophic phase inversion method.[11] The authors reported that each oil drop of would have undergone its own super saturation and nucleation process thereby producing large droplet size distribution.

Effect of Mixing Speed and SOR on Zeta Potential of Nanoemulsion

Zeta potential values have a vital role in emulsion stability and functional attributes. Zeta potential of nanoemulsions depend on various factors viz. surfactant type and concentration, system composition, pH, and electrolyte concentration.[17] In the current investigation, all the nanoemulsions samples obtained negative zeta potential values (Table 4). The

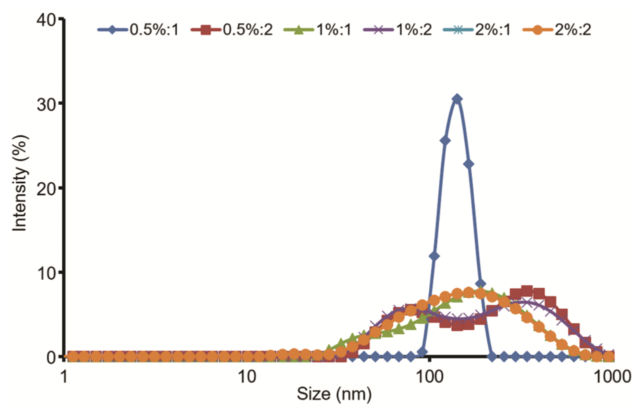

Fig. 1 — Droplet size distribution of olive oil nanoemulsions prepared using Tween 80 at different oil (%): SOR (0.5:1; 0.5:2; 1:1; 1:2; 2:1 and 2:2) employing a mixing speed of 1500 rpm

Table 3 — Polydispersity index (PDI) of olive oil nanoemulsions prepared using Tween 80

Mixing speed (rpm)	Olive oil (%w/w): SOR					
	0.5:1	0.5:2	1:1	1:2	2:1	2:2
500	0.67^{dB}±0.13	0.63^{cC}±0.17	0.60^{cB}±0.17	0.55^{bC}±0.05	0.53^{bB}±0.21	0.50^{aC}±0.14
1000	0.61^{cA}±0.11	0.58^{cB}±0.12	0.51^{bA}±0.13	0.49^{abB}±0.07	0.48^{aA}±0.13	0.46^{aB}±0.17
1500	0.59^{dA}±0.05	0.52^{cA}±0.09	0.49^{bcA}±0.03	0.41^{aA}±0.02	0.47^{bA}±0.04	0.43^{aA}±0.03

Mean ± Standard error (n=3); Significant differences in mean values ($p<0.05$) are indicated by different superscripts in the row (a,b,c,d,e,f) and column (A,B,C)

Table 4 — Zeta potential (mV) of olive oil nanoemulsions prepared using Tween 80

Mixing speed (rpm)	Olive oil(%w/w): SOR					
	0.5:1	0.5:2	1:1	1:2	2:1	2:2
500	$-17.20^{aA} \pm 1.94$	$-10.99^{bA} \pm 0.26$	$-8.86^{cA} \pm 0.05$	$-7.92^{dB} \pm 1.33$	$-10.31^{bA} \pm 0.50$	$-7.40^{dA} \pm 1.54$
1000	$-18.38^{aA} \pm 1.31$	$-8.52^{dB} \pm 0.16$	$-9.36^{cA} \pm 1.07$	$-11.8^{bA} \pm 0.10$	$-10.14^{bA} \pm 0.88$	$-8.73^{dA} \pm 0.15$
1500	$-10.5^{aB} \pm 0.70$	$-9.46^{bA} \pm 0.39$	$-7.60^{dB} \pm 0.52$	$-10.60^{aA} \pm 0.10$	$-10.28^{aA} \pm 3.74$	$-8.60^{cA} \pm 0.76$

Mean ± Standard error (n = 3); Significant differences in mean values ($p<0.05$) are indicated by different superscripts in the row (a,b,c,d,e,f) and column (A,B,C)

nanoemulsion droplets prepared by Tween 80 obtained net negative charge due to its non-ionic nature. The reason behind this could be due to the impurities present in the oil phase or due to the interaction between oxy-ethylene group of Tween 80 and water molecules in the occurrence of hydroxyl ion at the oil/water interface.[18,19] It was observed in the current study that zeta potential values of nanoemulsions were also influenced by oil concentration and SOR. At SOR 1, zeta potential value of Tween 80 nanoemulsions decreased when oil concentration rose from 0.5 to 1% and later zeta potential values increased when oil concentration further enhanced to 2%. However, at SOR 2 the zeta potential value increased when oil concentration rose from 0.5 to 1% and then reduced when oil concentration further increased to 2%. The packing of surfactant on oil surface depends on its molecular structure. It was postulated that zeta potential may increase with the surfactant concentration until saturation of surfactant at interface occurs, after that it may decrease further.[20]

Selection of Best Combination of Olive Oil Nanoemulsion Matrix

The best combination of olive oil nanoemulsion matrix was based on smallest droplet size and PDI values. Overall, the smallest droplet size was obtained in case of emulsion matrix combination with olive oil (2%), SOR 2 at the mixing speed of 1500 rpm.

Electrical Conductivity

The estimation of changes in electrical conductivity was done to confirm the phase inversion from w/o to o/w (water-in-oil to oil-in-water) with the progressive addition of aqueous phase in oil-surfactant mixture. Fig. 2 shows change in electric conductivity in olive oil-in-water nanoemulsion. There was occurrence of phase changes at 20% water concentration, whereas at low water content (10%) the w/o emulsion mainly found.[21] The viscosity of the system suddenly increased due to formation of gel-like structure. To dissolve this gel structure more addition of aqueous phase was required thus subsequently led to the preparation of o/w nanoemulsions. The main factors responsible for phase inversion are oil and aqueous

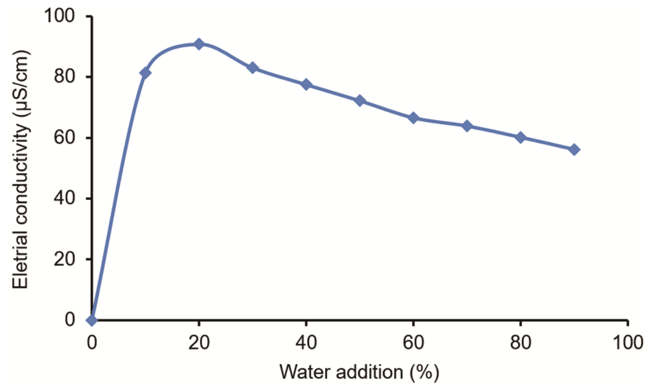

Fig. 2 — Changes in the electrical conductivity characteristics of olive oil-in-water nanoemulsion (2% oil phase, SOR 2) and mixing speed of 1500 rpm

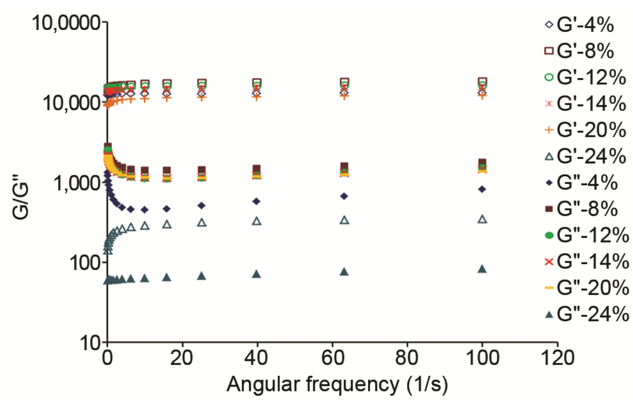

Fig. 3 — Changes in the rheological characteristics of olive oil-in-water nanoemulsion (2%), SOR-2 at 1500 rpm

phase ratio, collision between surfactant and aqueous phase. The surfactant characteristics, surfactant to oil ratio, surfactant affinity for the hydrophobic phase may be the key factors mainly responsible for collision between surfactant and aqueous phase.[14]

Rheological Characteristics of Olive Oil Nanoemulsion Prepared by Optimized Combination of Process Variables

Changes in rheology i.e. viscous behavior (loss modulus G'') and elastic behavior (storage modulus G') of nanoemulsion during its preparation are shown in Fig. 3. Rheological measurement of emulsion components oil-surfactant-aqueous phase is important as it determines the rate of transformation from w/o to o/w/o to o/w with addition of aqueous phase during phase inversion and hence the droplet size.[22] In the current study, the rheological behavior of oil-surfactant-aqueous phase system was determined at different levels of water addition (4, 8, 12, 16, 20 and 24%) during phase inversion process. The loss modulus value of emulsion mixture increased suddenly when the water addition increased from 4 to 8% and further water addition (upto 20%) led to slow and non-significant decrease in loss modulus value. When the water addition increased to 24%, sudden and significant drop in loss modulus & storage modulus value was observed. It may be termed as critical water concentration (20%) where lamellar crystalline phase o/w/o exists. This is supposed to be the point where o/w/o approaches their highest volume of water addition, just before the catastrophic inversion.[22,23] After the water addition (20%) o/w started to form and existed with o/w/o. The emulsion existed in the form of w/o below the water content of 8% after that intermediate multiple emulsions (o/w/o) formed. As further water addition increased o/w/o vanished and o/w formed completely. It is a sequential step that was believed to happen during the phase inversion process. In the current study, after 24% water addition the G' and G'' drastically decreased. Similar investigation has been made for the D-limonene nanoemulsion and soybean oil nanoemulsion prepared with EPI method wherein the critical water concentration reported to be 27% and 30%, respectively.[24, 25]

Effect of Change in pH, Salt Concentration, and Temperature Condition on the Stability of Olive Oil Nanoemulsion

The optimized nanoemulsion formulation i.e. olive oil (2%), SOR 2 and mixing speed of 1500 rpm having smaller droplet size and PDI value was chosen for this environmental stability test. To assess the stability of the nanoemulsion made with the best combination of process factors, it was exposed to a variety of environmental stress conditions, such as heating, salt concentration, and pH for commercial utilization in food industries. The optimized nanoemulsion exhibited a zeta potential of −8.60 mV and a droplet size of 131.47 nm. The size of the nanoemulsion was not significantly affected ($p>0.05$) with the change in salt concentration from 0.1 to 1.0 M. However, the salt screened the ions on oil droplets, the addition of salt produced lower zeta potential values (Table 5). The electrostatic repulsive forces between oil droplets were sufficient to keep them from coalescing. Tween 80 nanoemulsions were shown to be stable at increased salt concentrations.[9,26] The authors advocated that mixing is required to facilitate the surfactant movement to oil-water inter phase and to promotes the disorder of bicontinuous microemulsion occurred at the boundary of the oil and aqueous phase to obtain smaller droplets. The authors advocated that the dehydration of Tween 80's hydrophilic polymeric head groups could be the cause of the nanoemulsions decreased zeta potential with the addition of salt. These changes might have led to alterations in curvature of surfactant monolayer present on oil droplets leading to droplet coalescence.

High temperature (pasteurization, hot filling, and autoclaving) is often required during food processing condition. As heat treatment was raised from 65°C/30 min to 121°C/15 min, the mean droplet size of nanoemulsions increased (Table 5). The severe heat treatments used in this study (95°C/15 min and 121°C/15 min) might be the cause of the larger droplet

Table 5 — Effect of salt, temperature and pH on droplet size and zeta potential of olive oil nanoemulsion with olive oil (2%), SOR2 and mixing speed of 1500 rpm

Parameters		Droplet size (nm)	Zeta potential (mV)
Control*		$131.47^{a} \pm 2.13$	$-8.60^{a} \pm 0.76$
Salt (NaCl) concentration (M)	0.1	$151.50^{b} \pm 0.40$	$-1.34^{c} \pm 4.98$
	0.5	$151.73^{b} \pm 0.20$	$-2.87^{b} \pm 0.26$
	1.0	$148.75^{b} \pm 0.10$	$-2.30^{b} \pm 0.01$
Control*		$131.47^{a} \pm 2.13$	$-8.60^{b} \pm 0.76$
	65/30	$140.12^{b} \pm 0.11$	$-9.11^{a} \pm 0.11$
Temperature (ºC)/Time (min)	95/15	$153.91^{c} \pm 3.21$	$-6.41^{c} \pm 0.35$
	121/15	$165.55^{d} \pm 4.94$	$-6.87^{c} \pm 0.69$
Control*	6.71±0.41	$131.47^{a} \pm 2.13$	$-8.60^{a} \pm 0.76$
	1	$149.38^{d} \pm 0.34$	$-4.22^{c} \pm 0.14$
pH	3	$143.99^{c} \pm 0.1$	$-5.34^{b} \pm 0.25$
	7	$139.09^{b} \pm 0.94$	$-9.23^{a} \pm 0.22$

Mean ± Standard error (n = 3); Significant differences in mean values ($p<0.05$) are indicated by different superscripts in the column (a,b,c)

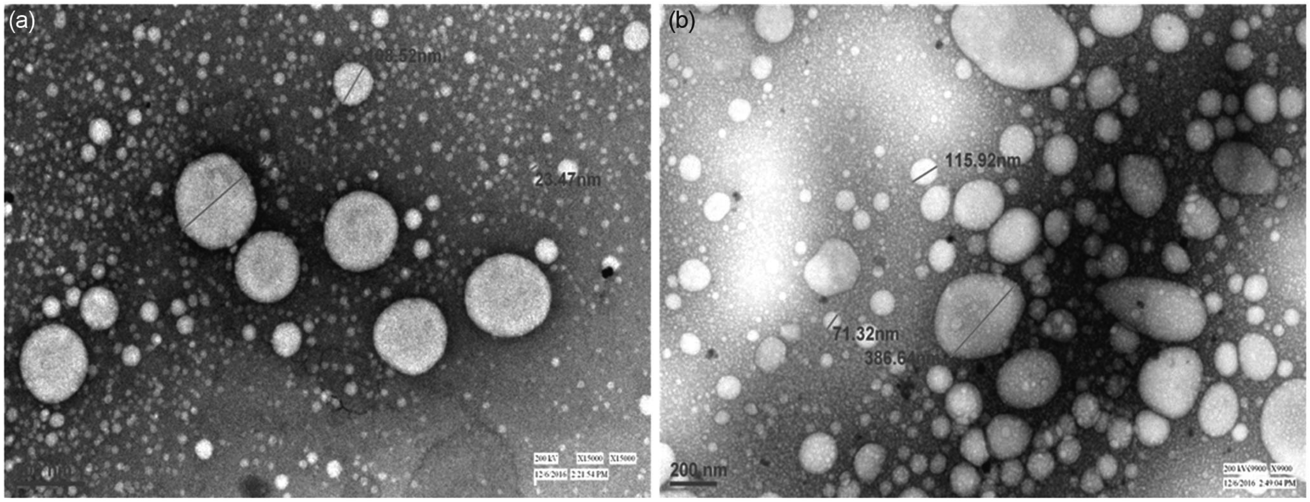

Fig. 4 — Changes in microstructure of olive-oil-water nanoemulsion (2% oil phase, SOR-2 and 1500 rpm) at (a) 0^{th} and (b) 30^{th} day storage duration at 4°C

size. Since Tween 80 undergoes phase inversion at 80°C, the larger droplet size at higher temperatures might be explained by increased droplet coalescence.[27] Heating the nanoemulsions to temperatures between 30 and 90°C did not significantly alter the size of the SDS (sodium dodecyl sulphate) and saponin stabilized nanoemulsion particles.[26] These findings showed that there was comparatively substantial electrostatic repulsion between the droplets, heat treatment had no effect on the surfactant monolayer's ideal curvature. As the temperature rose from 65 to 95°C, the zeta potential value dropped. Heat treatment at 70°C had the lowest zeta potential, and as temperatures rose, decreasing zeta potential values were seen.[28] When the pH dropped from 7 to 1, the zeta potential value of nanoemulsions considerably decreased ($p<0.05$), leading to a substantial increase in droplet size ($p<0.05$). However, in the case of droplet size, the amount of change in zeta potential values was mirrored. The types of emulsifiers utilized determine the nanoemulsions pH dependence. There was no droplet aggregation was observed for Tween 80, Quillaja saponin and SDS stabilized nanoemulsions as the pH altered from 2 to 8.[(27)] Higher zeta potential values of magnetite-based nanofluid had outstanding stability at room temperature with a slight degree of aggregation after 128 days without changing in appearance.[29] Tween 80 nanoemulsion was stable over a broad pH range because it was primarily stabilized by steric repulsion.[30]

Effect of Storage on the Microstructure Stability of Olive-oil-water Nanoemulsion

Transmission electron microscopy is a valuable technique to study the microstructure of nanoemulsion. It is one of the most popular microscopic methods for investigating the microscopic characteristics of colloidal systems which offers a thorough picture of the system at high resolution, including the microstructure and droplet size distribution of dispersed particles.[31] The changes in droplet size was found in the optimized nanoemulsion stored at 4°C for 30 days (Fig. 4). The TEM image at 0^{th} day of storage (Fig. 4a) indicated predominantly spherical and well-dispersed oil droplets. Most of the droplet sizes were in the nanometer range with diameters below 200 nm (range 23.47–227.78 nm). Also, the droplet surface is smooth with distinct boundaries between each droplet. This suggested effective interfacial coverage of emulsifier. After 30 days of storage, significant structural changes were observed. The TEM image (Fig. 4b) revealed that there was an increase in average droplet size, with some droplets exceeding the initial nano-scale range (71.32–386.64 nm). Partial droplet clustering could be clearly seen suggesting onset of destabilization mechanisms. Also, elongated droplet shapes indicated droplet deformation and possible coalescence. The broader size distribution reflected a reduction in uniformity compared to the freshly prepared sample. These findings were in accordance with findings of a study wherein soybean oil emulsions stored at 4°C for 3 months resulting large droplets greater than 1000 nm due to the destabilization mechanisms viz. flocculation and coalescence.[32] TEM analysis indicated that mean number-weighted size (dn) and median size of the droplets decrease during storage while larger droplets appear more predominant owing to Ostwald ripening in perfluorocarbon nanoemulsions.[33] The microstructure stability of DHA (docosahexaenoic acid) nanoemulsion was found stable towards droplet aggregation and it was successfully incorporated in apple juice for fortification.[34]

Conclusions

Low-energy emulsification was effectively found to produce nanoemulsion particularly at surfactant-oil ratio 2. Upon increasing the mixing speed from 500-1500 rpm, the droplet size decreased. The lowest droplet size was obtained 131.47 ± 2.13 nm at olive oil concentration (2%), SOR 2 and mixing speed of 1500 rpm. There was no drastic change in droplet size of nanoemulsion due to environmental stress condition. The droplet sizerange was 23.47-227.78 nm at 0^{th} day and changed to 71.32-386.64nm after one month storage as seen through TEM. It can be concluded from above results that olive oil nanoemulsion was reasonably stable to environmental stress condition and storage, which is advantageous for industrial use. The lipophilic bioactive compounds present in the olive oil can be preserved in form of nanoemulsions for various food applications using low-energy emulsification techniques. Additionally, the effect of processing variables for the formation of stable nanoemulsion would be helpful in designing lipophilic bioactive components such as vitamins, pigments, flavours etc. which broadens its utilization in food industries.

Conflict of Interests

None

Acknowledgement

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