Enhancement of food safety using nanoemulsion with emphasize on fish food: A Review

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Abstract

Nanotechnology is an innovative approach that its application has inspiring prospective for controlling and preventing diseases, extending shelf-life of food stuff, and other uses in biology, chemistry or industries. Nanoemulsions are products of a branch of nanotechnology comprising submicron emulsion that also refer to nanoemulsion (r < 100 nm) of which high energy as one of the nanoemulsion production method, includes rotary-stator mixers, high pressure homogenizer, microfluidization, ultrasound and membrane emulsion. Another one, low energy method is classified into isothermal and thermal. Isothermal method is, spontaneous emulsification, solvent displacement, and emulsion phase inversion. Also, from among the thermal methods it can be referred to the most important one of them which is the phase inversion temperature. In the following, the technics for recognition and diagnosis of nanoemulsion structures are discussed and these technics are generally divided into separation, physical properties determination, and imaging technics.

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Introduction

Nanotechnology is an innovative approach that its application has inspiring prospective for controlling and preventing diseases, extending shelf-life of food stuff, and other uses in biology, chemistry, biochemistry where this property is mostly contributed to atomic scale in the size range of 100 nm or smaller matters (Wang, Su, Nie, Sun, Zhang, Wu & Moustaid-Moussa 2014). The convergence of nanotechnology,in accordance with its many capabilities, and food science has led to more than 200 large worldwide companies investing in new products in the field of nanotechnology. Based on the tremendous potential for nanotechnology applications in the food industry, it is expected that this technology will initiate a major revolution in food and agricultural products that will have positive implications for the worldwide expansion of mechanized agriculture (Ahari, Hedayati, Akbari-Adergani, Kakoolaki, Hosseini & Anvar 2017). Among this technology, Nanoemulsions are the products of a branch of nanotechnology comprising submicron emulsion that also refer to nanoemulsion (r < 100 nm) have a number of possible benefits vs typical emulsions for better distribution and transfer of lipophilic compound in diet and other drinks with high visual transparency; high physical durability and improved bioavailability (McClements, Decker & Weiss 2007; McClements 2011).

Lipid structures can cover the medicines against breakdown courses. Accordingly, nanoemulsions in which the drug is injected in the interior period of the system protected from the exterior media. Nanoemulsions encompass a liquid stage, emulsifying compound and a combination of several oils (e.g. corn oil, mineral oil and olive oil) (Ferreira-Nunes, Gratieri, Gelfuso, & Cunha-Filho 2018). Nano-emulsions are also utilized for micro capsulation of bioactive compounds. Since bioactive compounds undergo undesirable changes in their properties after entering the body and going through digestion phases, as a result of change of the temperature, humidity, and pH, until the time they reach their target area in the body, they are stored in capsules smaller than 100 nanometers (Ahari 2017). As bioactive compounds contain aldehyde, ketone, ester bonds in their structure, they are great prone to oxidative decay, while micro capsulation prevents light and oxidative putrefaction and helps conservation of bioactive compounds without influencing the taste and texture of the food. They meanwhile improve the stability, solvability and release conditions of these compounds in the final product (Barani, Ahari & Bazgir 2018).

Like ordinary emulsions, However nanoemulsions are compounds with appropriate stability, pharmacodynamics and pharmacokinetic potential (Bali, Ali & Ali 2010). The unique structure of nanoemulsions compared with ordinary emulsions presented advantages for industries in particular food safety branch to extend use of it (Sajjadi 2006). In the present article, we review the methods of nanoemulsion production generally categorized as two groups of high-energy and low-energy production methods. The low-energy technique was studied against a high-energy technique (microfluidization). Minor droplets (d< 160 nm) can be formed by both methods, but less surfactant was required for the high-energy technique than the others (Ostertag, Weiss & McClements 2012).

In general, four components: (a) oil stage (b) water stage (c) surfactant and (d) energy needed to generate nanoemulsion (Figure 1).

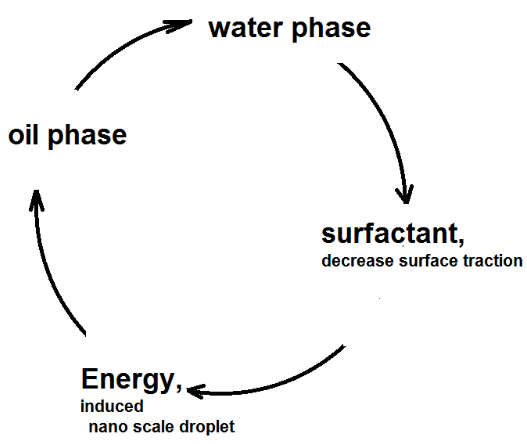

Figure 1. component in the generation of nanoemulsion

Dispersion-base Nanoemulsion

Based on their dispersion issue, emulsions are divided into two groups: Oil in water (O/W) and water in oil (W/O). In the former state, oil drops are dispersed in the water phase; at low temperature, the surfactant monolayer has a great positive impulsive curving fabrication of oil-swollen aggregated amphipathic solution phases (or O/W nanoemulsions) which may co-occur with an additional oil phase. At high temperatures, the impulsive curving turns into negative and water swollen reverse aggregated amphipathics (or W/O microemulsions) co-occur with additional water phase (Solans, Izquierdo, Nolla, Azemar & Garcia-Celma 2005).

For the instance, Milk is an instance of oil in water emulsion and water in oil is the reverse of oil in water emulsion (Li, Ma & Cui 2014). Butter and margarine are of this emulsion type.

Emulsions are also classified based on their layers. In multi-layer or double-layer emulsion, the drops of a dispersed liquid (which can be an emulsion, a micro-emulsion and a liposome), is dispersed once more in another liquid (which can be either water or oil), and in this way, a double-layer emulsion is formed. The double-layer emulsion is in two forms of water in oil in water (W/O/W) and oil in water in oil (O/W/O). In a study multiple W/O/W nanoemulsions were improved to fabricate skin use of medicine Aciclovir to fight against virus. To produce such this drugs, the phase inversion temperature (PIT) method was used to finalize the designs with no requirement to high pressure to be in (Schwarz, Klang, Karall, Mahrhauser, Resch & Valenta 2012).

The structure of these double-layer emulsions entails an emulsifier layer, too. (Saifullah, Ahsan & Shishir 2016).

Ordinary emulsions

The ordinary emulsion with a high light dispersion which is known as macro-emulsion, includes drops with an average diameter of 100 micrometers to 100 nanometers has unstable thermodynamic properties, and opaque in appearance. Nano-emulsions can be considered as ordinary emulsions which have very small particles with the average diameter of 20 to 100 nanometers. Their relatively small size in comparison with light wavelength makes the emulsion clear or a little opaque ($d<\lambda$), in other words, they scatter the light less and their small particle size makes them show a high resistance to collection and gravity separation; however, this system is still thermodynamically unstable. Micro-emulsion is thermodynamically stable and the average diameter of its particles ranges from 5 to 50 nanometers. As these particles are much smaller than light wavelength ($d<\lambda$), this emulsion is transparent and light dispersion is very little in it (McClements 2010). Due to the excellent characteristics that nano-emulsions have in comparison with ordinary emulsions, such as high stability, increase in surface tension in oil and water, high biological access, informality and safety, they have many applications in food industry including packaging (Hossaini, Asadnezhad, Ahari, Anvar, Abdi, Toumari & Dastmalchi 2014), processing, protecting and release of bioactive lipophilic materials (vitamins, pesticides, antimicrobial compounds, and antioxidants)

(Shahbazzadeh, Ahari, Rahimi, Dastmalchi, Soltani, Fotovat, Rahmannya & Khorasani 2009).

1. Nano-emulsions Ingredients

1.1. Oil Phase

Oil phase is composed of bioactive compounds such as fish oil, essential oils, oil flavors, and vitamins which are solved in carrier oil. Generally, different kinds of oil are accompanying with production of nanoemulsions, including (mono-di-tri) acyl glycerol, free fatty acid, essential oils, organic oils, as well as waxes among which triglycerides have great usages due to their low cost, nontoxicity, and abundance of their raw sources (soy, sunflower, corn, and canola oil). Considering the complex structure of oils, their polarity and viscosity features are often varied and this can affect the formation and stability of nanoemulsions. For example, vegetable oils usually have low polarity but their viscosity is relatively high. Thus, they were not used in the production of nanoemulsions using Phase Inversion Temperature (PIT) and homogenizer with high pressure (Jin, Xu, Liang, Li, Liu & Li 2016).

1.2. Water Phase

Water phase is one of the irreplaceable ingredients in the production of nanoemulsion. It can be explained that the proportion of water to oil phase is an important factor in the formation and stability of nanoemulsion. The other materials which can exist in the water phase are polysaccharides, cosurfactants, proteins, salts, and nutritious materials. These are important as the existence of compounds such as salts in the water could change pH, ionic structure, polarity and surface tension. Therefore, the liquid phase ingredients can be a decisive factor in determining the physicochemical properties of the nanoemulsion and the existence of materials such as proteins, pectin, agar resin, alginate, and other polysaccharides could help the stability of nanoemulsion through enhancing the viscosity. Regarding the proteins which are insoluble in water, ingredients such as soy and casein proteins are often used as surfactant (Qian & McClements 2011).

1.3. Stabilizers

As mentioned before, nanoemulsions are thermodynamically unstable; hence, their formation requires stabilizers. Emulsifiers are one kind of stabilizer, which has application in formation of tiny droplets during homogenization; meanwhile, they prevent the aggregation of droplets either during or after homogenization. Another kind of stabilizers used as a texture modifier, prohibits gravity separation and Oswald ripening in nanoemulsion (McClements & Rao 2011).

1.4. Emulsifier

Emulsifiers are materials which are active on the surface and have amplification property and have both lipophilic and hydrophilic compartments in their structure. Hence, one part is dependent on the nonpolar (oil) phase, and the other part on the polar (water) phase (Qian & McClements 2011). An important index which is employed for determining the dependence of emulsifier to polar and nonpolar phases is the HLB index which explains the ratio of hydrophilic to lipophilic groups in the molecule. If the HLB number is greater than 10, the surfactant dependence to oil phase becomes higher (lipophilic) and emulsifier is absorbed on the surface between water and oil and prevents the aggregation of oil droplets. Additionally, the decrease in surface tension increases the dispersity of droplets during homogenization. Also, the increase in surfactant thickness due to an increase in droplets surface and decrease in their sizes has been applied in ordinary emulsion (Yuan, Gao, Zhao, & Mao 2008). Meanwhile, emulsifier is selected based on the oil phase properties in the production of nanoemulsions. Nevertheless, proteins have tendency to form larger droplets.

1.5. Texture Modifier

Hydrocolloids have much influence on the thickening or jellification of texture in water phase. In other words, rheological modification of texture in water phase is very effective in stabilization of nanoemulsion against gravitational separation. Hydrated polysaccharides which have a vast structure, have many applications as thickeners, but polysaccharides with ampiphilic structure are more used as emulsifiers (Kumar, Ramalingam, Dasgupta & Ranjan 2016). In general, texture modifier is a compound that is characteristically added to the continuous step of nanoemulsion fabrication to alter its structure characteristics and to affect as a thickening object (McClements & Jafari 2018).

1.6. Weighting agents

These compounds are added to the dispersed phase to modify droplets density with the density of continuous phase. The goal is decreasing the impulsion force of gravity and delaying creaming or sedimentation (Salvia-Trujillo, Soliva-Fortuny, Rojas-Graü, McClements & Martín-Belloso 2017).

1.7. Ostwald ripening phenomenon delayer

One of the common challenges in the production of nanoemulsions from essential oils is overwhelming accumulation tendency of droplets after the time due to Oswald ripening phenomenon. In this phenomenon, oil droplets grow in the presence of water phase due to the difference in the chemical potential inside oil droplets, which have different sizes and ultimately this leads to separate phases. This phenomenon happens in oils such as essential oils, so that these oils, despite having hydrophobic properties, have a low tendency to solve in water phase. This problem can be delayed by mixing them with oils which are totally insoluble in water. In other words, ingredients with high hydrophobic property can be added to oil phase as a delayer of Oswald phenomenon (Ryu, McClements, Corradini & McLandsborough 2018).

2. Methods of nanoemulsion production

Fabrication of nanoemulsions is generally done through two methods, high energy and low energy. In the former, some factors for creating high mechanical energy are used to form particles in nano scale size such as temperature, time, and properties of ingredients. In the latter, low energy is used for forming nanoemulsion particles and the desirability of the size of particles (Ostertag et al., 2012), which are formed depends on the physical and chemical properties of surfactant and co-surfactant as well as the oil droplets, which were involved in the formation of nanoemulsion. Also, in low energy methods, the formation of nanoemulsion is dependent on the change of surface tension during phase to be changed (Cardoso-Ugarte, López-Malo & Jiménez-Munguía 2016).

2.1. High Energy Methods

2.1.1. Rotor-stator mixers

This method is widely used in a range of industries such as food, medicine, and cosmetics and is a standard method, which used for producing mid to high viscosity emulsions. On the top part of this device, the rotor or the twisting part is placed (Wooster, Andrews & Sanguansri 2017), which is jointed on an axis so that the part is protruded out of stator plate and liquid enters the rotor through the axis. Rotor accelerates the liquid flow first in a tangent form and then, it is conducted towards stator plate, which has a blade. Then, it is fed through the slot from plate to the stator (Scholz & Keck 2015). Some studies show harsh hydrodynamic tensions either in form of speed gradient or high chaos existed in the inner and the end part of the outer slot (van der Schaaf & Karbstein 2018). Hydrodynamic harshness and following that, the final particles size depends on the spin speed of the rotor in the industry. The passage flow is very slow and this is due to the fact that each drop of liquid must turn into a significant number of stable drops in nano-scale sizes (Håkansson & Rayner 2018).

2.1.2. High Pressure Valve Homogenization

This method is one of the very common methods in the industry, which is often used to minimize emulsion droplets using rotor mixer. Generally, this method entails two stages. In the first stage, the surface to volume ratio of the particles increases because of the decrease in their size and in the second stage, they become stabilized with the absorption of emulsifier in the interface. Samples in nanometer size can be produced with this device (Donsì, Sessa & Ferrari 2011; Lee & Norton 2013). The pre-emulsified liquid is by pump force and through valves with 10-100 micrometer slots under high pressure being commonly around 50 to 200 MPa. As the liquid enters the valve, it increases and reaches around 100 m s^{-1}, which high local speed causes local pressure decrease to under evaporation point that in turn causes bubble gaps. These bubbles move downward through valve where they face a high local pressure and burst. This burst sends strong waves. When the current is passing through the narrow slot, it is linear and transient, but when it exits the narrow slot and reaches the large chamber, it makes the current a strong and turbulent jet. This phenomenon leads to the breakage of the drops and decrease of their size. A homogenizer with high pressure is very helpful, but it requires high pressure in order to form nanoemulsion particles (Yu, Cha, Wu, Xu, Qin, Li & Du 2018).

2.1.3. Microfluidization

This method is similar to homogenizer, with high pressure method as a driving force guiding the relatively large particle emulsion into a channel with a narrow flow which is often called reaction room. as soon as the current enters the channel, it is accelerated and instead of being turned into a turbulent current at the end of the channel, it is conducted to form circular currents, which congregate and cause impact zone to be formed at the end of the narrow channel (Mahdi Jafari, He & Bhandari 2006). This convergence of current with high entrance pressure, leads into the formation of nanoemulsion particles (Villalobos-Castillejos, Granillo-Guerrero, Leyva-Daniel, Alamilla-Beltrán, Gutiérrez-López, Monroy-Villagrana & Jafari 2018).

2.1.4. Ultrasonication

In high pressure homogenizer, microfluidizer, and rotor-stator methods, the emulsion accelerating force, which is essential for nanoemulsion, is made with rotor blades, but in ultrasonication, breaking macroemulsion is done with ultrasound waves. In doing so, waves are in 20 to 100 KHz range and these ultrasound waves cause cavitation and fragmentizes the particles. These vibrations are amplified, made efficient and transferred into a probe. Then, cavitation bubbles are made and move due to pressure fluctuations. When these bubbles reach each other, they have a great shear force which breaks the particles into nanoemulsion particles (Abbas, Hayat, Karangwa, Bashari & Zhang 2013). Ultrasonication devices are in two forms of Batch and Continuous. The Piezoelectric probes in batch devices are used by being placed into a chamber or reactor, while in continuous systems, this is employed by passing the current from one or more piezotransmitter (s) (McClements, 2015).

2.2. Low Energy Methods

In these methods, nanoemulsion is produced based on phase transfer as either the temperature is constant and materials change, or materials are constant and temperature changes. In other terms, low-energy methods are of two types: thermal and isothermal. In the latter, we need changes in structure for producing small droplets; in the former, we need changes in temperature in order to make the drops (Komaiko & McClements, 2016; Ostertag et al., 2012).

2.2.1. Isothermal Methods

2.2.1.1. Spontaneous Emulsification (SE)

This method is based on a number of physicochemical mechanisms; that is, as two liquids which are insoluble in each other while to be in touch. They make an emulsion without any external help (either in thermal or mechanical forms). In this method, oil phase is titrated in a container of water phase which can initially be buffer solution. In this case, if structure, temperature and mixer speed are appropriate, particles of less than one hundred nanometers size are formed (Ganachaud & Katz, 2005). oil phase needs to be homogenized in a way that it is mixed with oil phase and hydrophilic surfactant and then oil phase is titrated in the liquid phase (Schuh, Bruxel, & Teixeira 2014).

2.2.1.2. Emulsion Phase Inversion (EPI)

This method entails adding water phase to oil phase while stirring. Oil phase usually contains oil and surfactant. The mechanism in this method is the reverse of SE method as water phase is first titrated in oil phase and water in oil emulsion is made. By adding more water, the liquid transparent phase which is formed is thickened and makes the stirring bar change from continuous to rotational. Formation of liquid transparent phase is a middle stage of nanoemulsion production which results in the production of a bi-continuous micro-emulsion. (Bilbao-Sáinz, Avena-Bustillos, Wood, Williams, & McHugh, 2010). In general, the critical amount of water in this method depends on the stirring pace, speed of water addition, and surfactant thickness. Then, water phase is titrated in it with a controlled speed. Extra stirring can also happen for ensuring the breakage and homogenization. As EPI is an isothermal method, keeping temperature constant has an important impact on the long-term storage of nano-emulsion in this method. This method is also called phase inversion composition (PIC) in some sources (Ostertag et al., 2012).

2.2.2. Thermal Methods

In these methods, changes in temperature are needed for producing nanoemulsion.

2.2.2.1. Phase Inversion Temperature (PIT) Method

In this method, basically, in order to produce nanoemulsion, hydrophilic and nonionic surfactant, oil and water are used. This production happens in three important stages. First, hydrophilic surfactant is mixed with oil and water in the mixer. Then, the resulting mixture is heated up to a temperature around or more than phase inversion. Ultimately, the solution is rapidly cooled or is cooled using cold water so that oil nanoemulsion is formed in the water (Rao & McClements 2010) . The reason for the formation of these small oil drops in oil water surfactant could be due to the changes in physicochemical properties of surfactant during heating. In low temperature, the head part of surfactant is extensively hydrated which means that surfactant is generally hydrophilic and resides in water phase. In high temperature, surfactant head loses water and lipophilic property appears in it, then, it resides in oil phase. (Anton & Vandamme 2009).

When the mixture is cooled from PIT high to its low temperature, surfactant molecules change from lipophilic to hydrophilic state and hence, their tendency to oil phase changes to tendency to water phase. (Saberi, Fang & McClements 2015).

3. Antimicrobial activity of nanoemulsion on fish and other animals

Herbal or other chemical-loaded Nanoemulsion comprises many applied profits for increasing food safety specifically while it would be incorporating with omega-3 oils into foods (Walker, Decker & McClements 2015). A mixture of chitosan biopolymer, nanoclay and Rosemary essential oil was modified as an applicable bionanocomposite coverage. Its capability to enhance the shelf life of fresh fillet fish, *Hypophthalmichthys molitrix* kept in refrigerator. Results confirmed this nanocomposite could proficiently expand its acceptability for one week. The veneer clearly slow lipid degradation in the fillets and prohibited microbial deterioration during 2 weeks of preservation (Abdollahi, Rezaei & Farzi 2014). As a nutritional supplement, nano scale resveratrol is capable to decrease bacterial and chemical evils, extend the shelf-life of fresh rainbow trout fillet incorporated with sodium alginate kept at 4 ^{O}C. Concerning the acquired results, sodium alginate/nano scale resveratrol 0.2% represented the highest prohibition impression on retarding the bacterial growth and chemical alterations of trout fillets (Bazargani-Gilani 2018). Coconut covering fluid smoke nanoemulsion had qualifications of particle size 13.43 nm, which are a potential antibacterial for fresh fish. Application of 5% fluid smoke of Coconut nano-scale partcles, prolonged the fish freshness up to 24 h at 24 ^{O}C. This nanoemulsion retarded the bacterial growth as bacteriostat. The greatest antibacterial feature was implemented against coliforms (Saloko, Darmadji, Setiaji & Pranoto 2014). Antibacterial effects of essential oil of rosemary, Laurel and thyme nanoemulsions on the specifications of trout were studied at 2 ^{O}C. The freshness of trout fillets was continued more than two week. The application of nanoemulsions based on herbal extracts decreased the quantities of the biochemical factors and bacterial growth (Ozogul, Yuvka, Ucar, Durmus, Kösker, Öz, & Ozogul 2017). Against to ordinary emulsions veneer, citrus essential oil-loaded nanoemulsions coating was impressive effect on prohibition the bacterial growth and alteration in the biochemical factors of the silvery pomfret within refrigerating storage. The results showed prolonging the shelf life of the fillets about 2 weeks. These findings supposed that nanoemulsion veneer could be an applicable conservation compound for marine fishes (Wu, Wang, Hu, Chen, Liu & Ye 2016). The adding of jujube gum enhanced the conservative effect of nanoemulsions so that the film layer comprising 3.5% nanoemulsion and 12% jujube gum showed the optimum feature to stabilizing the specifications of caviar fish fillet for 2 weeks at cold storage. An remarkable growth in the amount of chemical and bacterial attributes was recorded by increasing of days, whereas the values of pH, and sensory factors of the fillet quality decreased ($p < 0.05$) (Gharibzahedi & Mohammadnabi 2017).

Antimicrobial properties of betel leaf essence nano-emulsion. Betel essence, as the scatter phase, distilled water as the continuous phase, and twin 20 nonionic surfactant as the emulsifier were used in nano-emulsion production through high energy ultrasound method which was formulated in six different volume ratios of oil to surfactant (1:1 to 1:6). The antimicrobial properties of nano-emulsions were measured in five positive Gram and negative Gram bacteria strains. Finally, they concluded that this formulation of nanoemulsions can affect as a natural antibacterial material (Roy & Guha 2018).

Lu et al. (2018) explored the antimicrobial properties of Citral oil essence nano-emulsion. This nano-emulsion was composed of Citral essence, doubled distilled water, a combination of surfactants (Span 85 and Brij 97), and a co-surfactant. Citral concentration was 10 percent in all of the samples, but hydrophileelipophile balance (HLB) of the surfactant mixture ranged from 2 to 12 in the samples. The antimicrobial properties of Citral were studied using disk diffusion assay on *staphylococcus aureus, Listeria monocytogenes, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa,* and *Enterococcus faecalis* bacteria. however, the antimicrobial results of nano-emulsion on *Listeria monocytogenes* and *S. aureus* were different from the rest of species as they had a wider inhibitory area in comparison with the other bacteria (Lu, Huang, Wang, Yeh, Tsai, Huang & Li 2018).

Noori, Zeynali, and Almasi (2018) studied the antimicrobial properties of ginger essence emulsion and nanoemulsion and sodium caseinate edible coating on fillet chicken breast. This nanoemulsion was prepared using Tween 80 nonionic surfactant, ginger essence and double distilled water in two stages. In the first stage, the conventional emulsion was prepared by gradual and constant addition of the essence and Tween 80 into water while shaking (3000 rpm) and then, the resulting emulsion was transformed into nanoemulsion using the ultrasound method. Polydispersity index (PDI) decreased after preparation of the nanoemulsion (from 0.584 to 0.222). The highest intensity peak of nano-emulsion in 57 nanometer area showed that the majority of drops turned into very small particles after applying the ultrasound energy. Bacterial growth inhibition test was performed using Agar well diffusion and on two pathogenic species, *L. Monocytogenes* and *S. Typhimurium*. The treatment of coating with 3 percent antibiotic, Gentamicin had the highest growth inhibition in each of the microbial species and the sample with edible coating along with emulsion and nano-emulsion with two different percentages (3 and 6 percent) had the smallest growth inhibition. When the edible coating is utilized with the 3% of emulsion, the growth inhibition in *L. Monocytogenes* and *S. Typhimurium* is 10.33 and 8.66 mm, respectively. When the 6 percent emulsion was utilized, antimicrobial properties in *L. Monocytogenes* significantly increased ($p<0.05$); while there was not much change in *S. Typhimurium* ($p>0.05$). The treatment containing edible coating along with nano-emulsion in both of the microbial species had a significantly larger inhibition growth in comparison to ordinary emulsion ($p<0.05$). (Noori et al., 2018).

Li, Chang, Saenger, and Deering (2017) studied the antimicrobial properties of Thymol oil essence nanoemulsion through Spontaneous emulsification. The oil phase contained Thymol essence solved in ethanol (as a co-solvent) and the water phase contained one of the sodium lauryl sulfate surfactants (anion), polysorbate 20 (nonionic), triton X-100 (nonionic), or Brij 85 (nonionic) solved in water. Three different volume ratios (essence: surfactant) were considered for each surfactant type (1:2, 1:5, 1:10) and using diffused light spectroscope, it was found that the polysorbate 20 and sodium lauryl sulfate (regardless of volume ratio) with the particle diameter of 200 nanometers is the most effective surfactant for the formation of particles in nano scale. Negative Zeta potential in sodium lauryl sulfate which is a ionic surfactant was expected, but it was unexpected of the nonionic surfactants. This might be due to the existence of hydroxyl ion on the drop surfaces or impurity in the reagents. Nano-emulsions stability was measured by measuring the Z-average, diameter and PDI after being exposed to heat and physical tensions and the size of particles containing polysurbate 20 and sodium lauryl sulfate did not change as a result of being centrifuged and facing cold and heat, but the samples containing other surfactants accompanying with an increase in size after being exposed to tension the samples with diameters more than 1000 nanometer was formed. Antimicrobial tests of thymol emulsion were first evaluated against *E. coli*, *E. faecalis*, and *Candida albicans* species in plankton state. The highest antimicrobial property concerning the samples, had surfactants of polysorbate 20 and sodium lauryl sulfate (with the lowest concentration). Emulsions were most effective on *E. coli* and had the least effect on *E. faecalis*. The contradiction which existed between the surfactant and essence was totally transparent in the sample containing Triton-X 100 in 1:2 and 1:5 mass ratios due to the lack of antimicrobial property. Generally, formulation without surfactant had the highest inhibition. (Li et al., 2017).

Hilbig, Ma, Davidson, Weiss, and Zhong (2016) did a research on the physical and antimicrobial properties of cinnamon bark essential oil (CBO) along with lauric arginate (LAE) which is a cation surfactant solved in water with antimicrobial property and Tween 80 nonionic surfactant. Cinnamon oil with 1% wt in water phase which contained Tween 80 or Tween 20 (3%wt) surfactant was combined with lauric arginate surfactant (0.05-0.375 weight percent) and ultimately, All of the samples in which Tween 20 was used, became opaque before and after heating. Contrarily, samples in which Tween 80 were used, transparent both before and after heating. In samples with Tween 80 (except for the samples with 0.05 %wt. of Lauric arginate) stability and no sediment was observed. In the samples with the least concentration of Lauric arginate, an increase in the hydrodynamic diameter was seen and in all of the samples, the hydrodynamic diameter decreased after 30 days of storage. However, this decrease was not statistically significant ($p>0.05$). There was no significant difference in the PDI of particles before and after one month storage ($p>0.05$). MIC index for *E. coli* and *S. enteritidis* along with the nanoemulsion was shown 1 ppm greater than the amount in Lauric anginate while 2 ppm greater in the nano-emulsion for *L. monocytogenes,* than that of in Lauric Arginate. In general, MBC was more than MIC and no growth inhibition was observed for Tween 80 up to 60.00ppm. MIC (400 ppm) and MBC (600 ppm) in cinnamon essential oil was the same for all bacteria. The presence of Lauric arginate with cinnamon essential oil against Gram negative bacteria, showed a decrease of CFU of bacteria as first and was along with an increase ultimately. In positive Gram bacteria, the only significant growth inhibition was occurred in the Lauric arginate and cinnamon essential oil combination. The greatest microbicideal affect occurred against *L.monocytogenes* in the media along with mixtureof lauric arginate and cinnamon essential oil after 4 h, in which the complete inhibition was performed. The nanoemulsion and pure Lauric arginate had the greatest growth inhibitions at 8 and 24 h, respectively. Regarding the mixture of Tween 80 and cinnamon essential oil, growth inhibition was low and reached under the diagnosis level (1 log CFU mL^{-1} after 48 h). Nanoemulsion effectiveness was a little lower, but the complete deactivation of bacteria occurred after 8 h. Tween 80 and cinnamon essential oil mixture had a slower inhibition as compared with the nanoemulsion, which occurred after 24 h. Lauric arginate alone had the least antimicrobial effect on *E. Coli* (3.5 log CFU mL^{-1}) after 24 h. For *L. monocytogenes*, no difference was observed between the effectiveness of the treatments (except lauric arginate alone) at 24, but after 48 h, in the treatment containing nanoemulsion, a 3 log CFU ml^{-1} of *L. monocytogenes* was observed but in that of Lauric arginate, the least effect was observed. Based on the results of this research, using Lauric arginate along with Tween 80 led into an increase in nanoemulsion stability without any side effect on its antimicrobial properties (Hilbig et al., 2016).

Other researchers (Liang, Xu, Shoemaker, Li, Zhong & Huang 2012) tried to produce peppermint oil (PO) nanoemulsion using homogenizer with high pressure to study its physical and antimicrobial properties. Water phase was achieved by combining pure gum powder in double distilled water in 21%wt. This study showed that the particles average diameter significantly decreases by increasing the homogenizer pressure from 50 to 100 MPa in 10 cycles but if pressure goes up, particles diameter cannot be changed. When the proportion (starch:oil phase) changed from 1:3 to 3:3, particles average size did not change significantly ($p>0.05$), but by increasing this proportion, particles average size significantly changed from 198 nanometers (3:3) to 228 nanometers (4:3). A small PDI indicated the narrow distribution of particle. Therefore, by increasing oil phase from 1:3 to 2:3, PDI significantly changed from 0.254 to 0.302 and by increasing this proportion. It was observed no changes in PDI, unless oil phase proportion exceeded 4:3. They ultimately concluded that if the proportion of oil phase to starch to be 3:3, the average diameter of particles would be smaller, and the distribution of particles would be narrower. The bacterial death time assay was accomplished in order to compare inhibition growth in emulsion without essential oil, pure essential oil, and essential oil nanoemulsion treatments. It was concluded that the emulsion without essential oil would not have any growth inhibition; while by adding peppermint essential oil, whether in pure form or as nano-emulsion, a great growth inhibition was observed for both species of bacteria. In *L. monocytogenes* during the first 8 hours, 10^1 CFU ml^{-1} bacteria were eradicated, but bacteria cells grew very fast after that time. This arising trend was slower in the nanoemulsion than in pure essential oil, so that after 36 hours, *L. monocytogenes* levels in essential oil and nano-emulsion were 10^7 and 10^4 CFU mL^{-1}, respectively. Regarding *S. aureus*, , only 10^1 CFU mL^{-1} growth bacteria prohibited during 3 h and this inhibition effect continued until 24 h for nanoemulsion. Ultimately, essential oil and essential oil with nanoemulsion decreased the amount of bacteria reached to 10^5 and 10^2 (CFU mL^{-1}), respectively (Liang et al., 2012).

Conclusion

Generally, there exists a tendency in the industries toward using natural materials with functions like artificial materials. The recent researches show the numerous advantages of nanoemulsions in providing bioactive and antimicrobial natural materials. The function of these materials are due to their small size and their high surface to volume ratio which cause more interaction of antimicrobial nanoemulsion with microbial cells or in nanoemulsions which contain bioactive materials, they increase the biological access and digestion ability of these emulsions. Despite all these advantages, there exists a need to more researches in this regard in order to make practical and industrial nanoparticles in particular, nanoemulsions. As an instance, in order for using nanoemulsions of oils, there should be further researches on their relationship with macro-molecules, carbohydrates, or fibers so that these products can supply the consumers and producers with valid data.

Conflict of interests

The authors declare that there is no conflict of interest.

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افزایش ایمنی مواد غذایی با استفاده از نانو امولسیون با تکیه بر غذای ماهی: مروری

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چکیده

فناوری نانو رویکردی نو آورانه است که به کاربرد آن در آینده، در جهت کنترل و پیشگیری از بیماری‌ها ، طولانی کردن ماندگاری مواد غذایی و سایر کاربردها در زیست شناسی، شیمی یا صنایع، امید بسیاری است. نانوامولسیون‌ها محصولی از فناوری نانو هستند که شامل امولسیون‌های زیر میکرون می‌باشند (r <۱۰۰). انرژی بالا به عنوان یکی از رویکردهای ساخت آن، شامل روش‌های میکسر روتار- استاتور، هموژنایزر فشار بالا، میکروفلودازیشن، مافوق صوت و امولسیون غشایی می‌باشد. رویکرد دیگر کم انرژی است که به دو گروه ایزوترمال و ترمال طبقه بندی می‌شود. روش ایزوترمال عبارت است از: امولسیون خودبخودی، جابجایی حلال و وارونگی فاز امولسیون و از بین روش‌های ترمال می‌توان به مهم‌ترین آن که روش دمای وارونگی فاز است اشاره کرد. در ادامه، تکنیک‌های شناسایی و تشخیص ساختارهای نانو امولسیون مورد بحث قرار گرفته و این تکنیک‌ها به طور کلی به روش‌های تفکیک، تعیین خواص فیزیکی و تکنیک‌های تصویر برداری تقسیم می‌شوند.

کلمات کلیدی: روش‌های انرژی بالا، روش‌های انرژی پایین، نانوامولسیون، خواص ضد میکروبی

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