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Review

Investigation of the effect of nanoemulsions in encapsulation of food ingredients, supplement and increasing their stability: a review article

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ABSTRACT

Emulsions with a nanoparticle diameter are called nanoemulsions. Nanoemulsions actually refer to dispersed systems of different liquids. Nanoemulsions contain oil, water, and an emulsifier. Nanoemulsions have more prominent properties (they are more stable against droplet aggregation and biphasic) than emulsions. Due to these properties, it is more appropriate to use them in some foods and supplements. Also, the advantages of this method and the use of food-grade compounds in their preparation is a safe and appropriate method for the production of encapsulated food compounds and supplements. Nanoemulsions can be used in different fields of food technology and food supplements by increasing bioavailability, release control and preservation of compounds. The activity of some food ingredients and supplements such as vitamins, enzymes, synthetic and natural antioxidants, colors, synthetic and natural antibacterial, etc. can be maintained by using nanoemulsions for a longer period of time. Therefore, in this article, we have tried to examine various aspects of nanoemulsions, including existing preparation techniques and their various applications in food industry technology and food supplements.

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1. Introduction

Today, the use of nanotechnology and control of the release of compounds in food is of particular importance. This discussion on food safety is also given much attention in order to maintain the health of the food, prevent the formation of harmful compounds and loss of food ingredients during storage in the food product (1). In order to protect the additives and functional compounds present in the food from adverse environmental conditions during processing and storage of the product, the use of encapsulation process for these compounds has recorded successful results in increasing the shelf life of the food product (2,3).

Encapsulation of food compounds can be performed in several techniques. One of these techniques is the nanoemulsion preparation technique, which has several functions. One of the most important applications of this technique is to increase the solubility of two heterogeneous liquid phases in each other. The use of nanoemulsions also increases bioavailability, preserves lipophilic compounds in the continuous aqueous phase, controlled release of hydrophobic food components and improves aroma, taste and texture (4,5).

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Emulsions are produced by mixing two immiscible phases with a surfactant or emulsifier that allows the two phases to be placed together. Then, using energy, their size is reduced and they become nanoemulsions (6,7). Over the past 10 years, the number of studies in the field of nanoemulsion enclosure technology has increased significantly. Due to the high Surface-area-to-volume ratio, nanoemulsions have good performance in the controlled release of food additives, on the other hand, as the size of particle decreases, the tendency of the compounds to bind to each other decreases. It can be successful in encapsulation. The size of nanoemulsions has been stated in the range of 10-200 nm (8,9). In order to produce nanoemulsions, different components are used to produce nanoemulsions. The first component, in many methods of producing nanoemulsions, requires solvents, which are often food grade due to their use in food . The second component used is surfactants or emulsifiers, which are used to reduce surface tension, the possibility of two dispersed and continuous phases to be placed next to each other and stabilize the produced nanoemulsions. Ionic, non-ionic and cationic surfactants and emulsifiers such as protein, polysaccharide and hydrocolloid are used for this purpose. Many surfactants and emulsifiers are used to encapsulate food ingredients, but the most commonly reported compounds in studies are Span 20, Tween80, pectin, concentrated whey protein, isolated whey protein, gelatin, and sodium caseinate. The third component, is a food additive with hydrophobic properties (10). Other components of this system are dispersed phase and continuous phase, which depending on the type of emulsion are usually continuous phase of water and dispersed phase of oil. In preparing nanoemulsions, less surfactant is needed than emulsions due to the reduction in size (11). The elements and important parameters of nanoemulsion encapsulation are defined in the section titled elements and parameters of nanoemulsions preparation for encapsulation of food constituents. The most important indicators during the preparation of nanoemulsions is to achieve high product efficiency with the highest encapsulation efficiency. Also, encapsulation of food additives with this method using multiple nanoemulsions can be used more widely.

Single nanoemulsions are limited and only have the ability to enclose hydrophobic or hydrophilic compounds alone, but sometimes due to the specific conditions of the encapsulated compound it is necessary to encapsulate two food additive compounds simultaneously. Therefore the use of double water-in-oil or oil-in-water-in-oil nanoemulsions is necessary (11-13). This topic has been the focus of published works in current years and therefore it is briefly discussed in the section titled double nanoemulsions. The aim of this study was to investigate the factors affecting the preservation of food ingredients, nanoemulsion and technique of production of nanoemulsion.

2. Elements and parameters of the preparation of nanoemulsion process for encapsulating food ingredients

The production of emulsions is performed by mixing two dispersed and continuous phases with an emulsifier. To reduce the size of the emulsion produced to the nano range, there are generally two methods with low energy and high energy, and each of these general methods is divided into more detailed methods (6,14,15). Low energy methods include phase inversion and solvent displacement, spontaneous production of nanoemulsions, and membrane emulsification (16-19). High-energy methods include high-pressure homogenizers, microfluidizers, and Ultrasonic homogenizers, which use energy-generating equipment to apply and generate sufficient energy (14,20-22). The following, are some of the methods described above.

2.1. High-energy approaches

2.1.1. High pressure homogenizer

In this method, the force generated by the high pressure homogenizer produces a nanoemulsion that may be affected by the control valve. In this way, increasing the temperature can reduce the stability of the produced nanoemulsions (23,24).

2.1.2. Ultrasonic homogenizers

In the ultrasound method, sound waves are used to reduce the size of emulsions produced in the nano range. Nanoemulsions produced by this method are usually more stable than other high energy methods. Another advantages of this method is the low production costs. The basis of ultrasounic method in reducing the size of emulsions is to create small cavities in their structure and thus reduce their size (25-29). Therefore, it can be said that using this method is more popular than other high-energy methods

2.1.3. Microfluidizer

In this method, first the emulsion is produced in larger dimensions and then by being in the microfluidizer homogenizer, the dimensions of the produced mixture are placed in the range of nanoemulsions. The nanoemulsions produced by this method are similar to high pressure homogenizer and have great stability, but the particle size is larger in this method (25,26).

2.2. Low-energy approaches

2.2.1. Membrane emulsification

In this method, using a dispersed phase membrane, it is spread in a continuous phase, although in this method, mechanical pressures used in the high-energy method are avoided and also less surfactant is used, but there are limitations such as larger size and lower stability of the produced nanoemulsions, which are affected by the stirring speed, the amount of surfactant, etc (24, 27,28).

2.2.2. Phase inversion

In the phase inversion method, water in oil emulsions, are produced using non-ionic surfactants that have the ability to change the amount of bond between water and oil and are produced by gradually increasing the amount of water (29,30). According to the contents, nanoemulsions produced by high-energy method, despite having mechanical effects, are able to produce more stable nanoemulsions with smaller dimensions.

3. Nanoemulsion encapsulation of different food bioactive

Nanoemulsification encapsulation is often used for hydrophobic food components such as carotenoids, polyphenols, essential oils, fat-soluble vitamins, fatty acids, flavonoids, and coenzyme Q10. In this study, we focus on encapsulation using nanoemulsions of several groups of bioactive food ingredients that have received much attention in current years.

4.Nanoemulsion encapsulation of essential oils

Antioxidant and antimicrobial compounds are a group of food additives that are sometimes naturally present in the food but are prone to decay due to lack of protection in the natural food system. On the other hand, these additives may react with other constituents of the food and lose their effectiveness (31,32). Also, essential oils strongly affect the sensory properties of food due to their strong flavor in food (33). The use of essential oils can be a good alternative to antioxidants and chemical antimicrobial compounds. But, due to low solubility in the aqueous environment, their use is limited, and encapsulation with nanoemulsions can be a good solution (34,35). Therefore, in order to increase the shelf life of the food and prevent the effect on the properties of the food, confinement of the mentioned compound is of special necessity. Nano emulsification has been proven to be a popular method for the protection of essential oils, and due to the lipophilic properties of these compounds, the use of nanoemulsions allows to maintain and control the release of essential oils well in aqueous matrix. Mostly essential oils have effective compounds with antioxidant and antimicrobial properties and also have the ability to inhibit oxidizing enzymes. Among these effective compounds in essential oils can be obtained carvacrol (40), allicin (made from garlic extract) (41), curcumin (42), cinnamaldehyde (43) and eugenol (44) and etc.

A group of oxidizing enzymes such as polyphenol oxidases are responsible for the enzymatic browning of compounds such as apple and apple juice, which in addition to reducing the apparent quality also reduces the nutritional value. On the other hand, the use of cinnamon extract, which has the property of inhibiting the mentioned enzyme, can be significantly effective in maintaining the quality of food products. Studies have shown that nanoemulsions with a concentration of 4% of this extract in apple juice still retain their color after 36 h of storage, and polyphenol oxidase activity has been shown to reduce its activity by almost zero (36). Other studies on nanoemulsions containing essential oil derived from cinnamon have shown that cinnamaldehyde also has an antimicrobial effect by acting on bacterial membranes, inhibiting ATPas and cytokinesis, and this effect on gram-positive bacteria is more than gram-negative (37). Further reports indicate that the use of ultrasound in the production of nanoemulsions containing cinnamon extract by increasing the stability and production of smaller particles by controlling the release of effective compounds has increased the antimicrobial effect of this compound during storage (34,38).

The effect of nanoemulsions containing oregano essential oil on the inhibition of microorganisms has shown that in the minimal inhibitory concentration (MIC), nanoemulsions containing oregano extract are not significantly different compared to free oregano essential oil. But, after storage for 3 h, nanoemulsions containing oregano essential oil significantly kill microorganisms, especially *Escherichia coli*, due to release control (39).

Another study on the antimicrobial activity of oregano essential oil showed that foodborne microorganisms such as *Salmonella typhimurium*, *Escherichia coli O157: H7* and *Listeria monocytogenes* increased the concentration of nanoemulsions from 0.05 to 0.1 % had a significant effect on the reduction of *Salmonella typhi*, however to reduce the number of *Listeria monocytogenes*, the concentration of 0.05% of this extract was sufficient (48). A summary of the recent works in this field is presented in table 1.

5.Nanoemulsion encapsulation of Omega-3

Fatty acids and omega-3 compounds are among the essential compounds for human health and in addition to having hydrophobic properties, they are very sensitive to environmental conditions (40). Due to the low consumption of these essential compounds by human, food fortification is especially necessary in this regard (41,42). Omega-3 compounds, which include the two polyunsaturated fatty acid; eicosapentaenoic acid (EPA) and decosahexaacetic acid (DHA), are predominantly found in fish oils. Therefore, the use of these compounds, even if you ignore the lipophilic properties and its sensitivity to adverse conditions, adding it directly will cause an inappropriate aroma and flavor in the food product (43,44). As a result, the combination of these factors, including hydrophobicity, hypersensitivity and unpleasant taste and odor, indicates the need to encapsulate omega-3 compounds and fatty acids. Encapsulation methods that use heat in their preparation cause damage to these compounds (45). Therefore, the use of nanoemulsions to solve these limitations is a good strategy and one of the advantages of this method is not using solvents, heat and harmful factors to fatty acids.

Thermal stability, pH protection, the presence of salts and the oxidative stability of anoemulsions containing omega-3 compounds, depend on the type of surfactant. In a study that used 4 types of emulsifiers, Tween 80, lecithin, sodium dodecyl sulfate (SDS) and saponin at acidic pH it showed that, Tween 80 is stable due to pH reduction to 2, while other emulsifiers such as lecithin accumulate due to acidification of the storage medium and lose their effect. Also, in terms of thermal stability if the pH is neutral and salt is not present, SDS and Saponin show the highest stability and the nanoparticle particles remain stable up to 90°C. Regarding oxidative stability, the lowest value of peroxide and by-products of oxidation was related to saponin (46).

Also in other comparisons, the use of tween 40 surfactants, soy lecithin and sodium caseinate in nanoemulsions of omega-3 fatty acids has been shown that the highest storage stability is related to nanoemulsions stored at 4°C. and oxidative stability show that nanoemulsions containing Tween 40 perform better than other surfactants and had a peroxide value of 1/2 to 1/3 times lower than nanoemulsions containing two other surfactants (47). Other factors affecting the oxidative stability of nanoemulsions containing omega-3 unsaturated fatty acids include the method of preparation of nanoemulsions , in case of using methods such as microfluidizers that transfer a lot of stress to nanoemulsions, an increase in oxidation rate has been observed in nanoemulsions (48). A summary of the recent works in this field is presented in table 2.

Table 1. A brief overview of some recent studies on nanoemulsion encapsulation of essential oils

Enclosed composition	Emulsifier	Nanoemulsion production method	Result	reference
Grapefruit peel extract	Tween 80	Ultrasound	Nanoemulsions of the extract did not have the effect of killing microorganisms but were able to inhibit the growth of microorganism.	**(64)**
Avocado peel extract	Lecithin	Solvent transfer	The results showed that the amount of nanoemulsions of the extract at a concentration of 50 µg/mL had an effective role in inhibiting cancer cells and the use of lecithin is very effective in the stability of nanoemulsions.	**(65)**
Grape Seed Extract	Lecithin	High pressure homogenizer	In the study, nanoemulsions based on sunflower oil (liquid) and palm oil (solid) were performed, which showed more stability of nanoemulsions based on sunflower oil, and also encapsulation significantly better antioxidant activity than the Unbound samples.	**(66)**

Table 2. A brief review of some recent studies on nanoemulsion encapsulation of unsaturated and omega-3 fatty acids

Enclosed composition	Emulsifier	Nanoemulsion production method	Result	reference
Unsaturated fatty acids Carp oil	Tween 80	High pressure homogenizer	The results show that the value of peroxide in the free state decreases from 3.6 to 15 after 49 days, while in nanoemulsions it increases from 3.6 to 6.	**(67)**
Flax seed oil	Saponin	High pressure homogenizer	The use of casein in the encapsulation of flaxseed oil provides better and more protection of its unsaturated fatty acids against oxidation and has an antioxidant effect	(68)
Flax seed oil and fish oil	Tween 80	High pressure homogenizer	examination of peroxide value in both flaxseed oil samples and fish oil samples shows that Nanoemulsions containing the desired oil with curcumin as a natural antioxidant compared to non-emulsified and non-curcumin samples, has a lower peroxide value after 30 days of storage.	**(69)**

6.Nanoliposome encapsulation fat-soluble vitamins and carotenoids

Fat-soluble vitamins contain vitamins K, D, A and E, which have a health role in the body and should be consumed through food. Although, the required amount of these nutrients for the body is small, but due to high sensitivity to environmental conditions, a large amount of these compounds are lost before the food reaches the consumer. Therefore, adding these nutrients to beverages and foods is of particular importance (49).

Carotenoids are also a group of natural dyes in plant foods that create a color spectrum from yellow to red depending on the structure of the food. On the other hand, carotenoids are of special importance in food because in addition to creating color in food, some of them act as antioxidants and the group that includes carotenes, in addition to antioxidant activity, are also precursors of vitamin A (50). Both of these compounds are prone to extinction due to sensitivity to environmental conditions, and on the other hand, due to the lipophilic behavior of these compounds, their use is limited in foods that have an aqueous matrix. Another challenge facing these compounds is their low bioavailability (51). It is important to use an appropriate encapsulation method to produce enriched compounds with improved stability of vitamins and carotenoids and to protect them. The use of encapsulation process using nanoemulsions is a process with a successful and safe approach due to their ability to encapsulate hydrophobic compounds and to be in the aqueous matrix. Factors such as pH, salt concentration, temperature and humidity are effective in the stability of nanoemulsions containing vitamins and reducing the amount of vitamins during storage. Vitamin D3 nanoemulsions have been reported to have the least loss and the most stability in a condition that the concentration of surfactant used in them was 20% and vitamin 30% .Studies on the stability of nanoemulsions in refrigerator, ambient and high temperature storage (60 °C) have shown that nanoemulsions are stable for 90 days at refrigerator temperature, 30 days at ambient temperature and 15 days at high temperature and also in acidic pH, the most degradation of vitamin D3 has been observed. The observations made in this study show that encapsulation using nanoemulsion significantly increases the storage time and preservation of the encapsulated material (52).

β-carotene, like other food components, is sensitive to environmental conditions, so studies on the factors affecting the stability of β-carotene nanoemulsions have been performed, which shows the effect of several factors, such as the use of several emulsifiers simultaneously (68) as well as it is a biopolymer such as chitosan (69). In a comparison between 3 samples of free beta-carotene, beta-carotene nanoemulsions with chitosan biopolymer showed that between 4 different temperatures of 4°C, 25°C, 37°C, 45°C, thermal stability of beta-carotene nanoemulsions with chitosan was higher than others (53). A summary of the recent research in this field is revealed in table 3.

7. Double nanoemulsions

Encapsulation using nanoemulsions is very popular due to the mentioned benefits and is also one of the newest methods of enclosing nutrient compounds. But these compounds individually only have the ability to enclose hydrophobic or hydrophilic compounds alone. However, in cases such as encapsulation of essential oils to enhance the desired effect, encapsulation with a hydrophilic antioxidant compound such as ascorbic acid can have a significantly positive effect (54).

On the other hand, sometimes the simultaneous confinement of two hydrophobic compounds together can cause adverse effects and by establishing a connection and reaction with each other, they eliminate the effect of the compounds and sometimes cause adverse and harmful effects (55). Therefore, according to the mentioned contents, the use of dual nanoemulsion structures will be important. In the preparation of dual emulsions, the presence of both hydrophilic and hydrophobic surfactants with hydrophilic lipophilic balance (HLB) index related to each phase is necessary, so in W/O/ W emulsions, the first emulsifier used has HLB close to zero and the second emulsifier according to the blue nature of the external continuous phase, it has more HLB and is close to 20 (56-58).

Table 3. A brief overview of some recent studies on nanoemulsion enclosures for carotenoids and vitamins

Enclosed composition	Emulsifier	Nanoemulsion production method	Result	reference
β-Carotene	Tween 20	High pressure homogenizer	The bioavailability of the samples was assessed after 24 h and shown that Nanoemulsion samples were significantly higher than free β-carotene	**(70)**
β-carotene Ascorbic acid α- tocopherol Ascorbyl palmitate	Tween 80 Span 80	High pressure homogenizer	The use of ascorbyl palmitate antioxidant compared to other beta-carotene antioxidants has resulted in lower peroxide value and beta-carotene loss during storage of nanoemulsions.	**(71)**
Astaxanthin	Tween 80	High pressure homogenizer	nanoemulsions produced with hypnotherapy emulsifiers showed less stability against acidic pH than tween 20 but were more stable against oxidation and heat increase.	**(72)**
Lycopene	Triglyceride	High pressure homogenizer	The use of modified starch in lycopene nanoemulsions has further reduced particle size and improved its stability	**(73)**
Tocopherol	Tween 80	High pressure homogenizer	The peroxide value of the samples shows that among the 3 concentrations of tocopherol 0, 250 and 500 mg/kg nanoemulsions, the concentration of 500 mg/kg after 12 days of storage is the lowest.	**(74)**
D_3	Tween 20 Lecithin	High pressure homogenizer	The resulting nanoemulsions were added to milk and shown to be resistant to particle growth for up to 10 days, depending on the concentration of vitamin D in the nanoemulsion.	**(75)**

In order to prepare multiple nanoemulsions, first the internal and intermediate phase emulsions are prepared and then the secondary emulsion. In the preparation of these emulsions, in the first stage, in order to increase the stability and reduce the particle size at the nanoscale, high energy is used and then less energy is used to prevent the internal droplets from disintegrating (59).
Nanoemulsion stability studies show that the type of emulsifier used is effective in the stability and maintenance of nanoemulsions, but it is important to note that sometimes the simultaneous use of two emulsifiers can further increase this stability (60). Another advantage of multiple emulsions is the increased stability of nanoemulsions due to the use of multiple biopolymers (61,62) . Regarding the factors related to the efficiency of multiple emulsions, we can mention the type of surfactant used, the method of preparation of nanoemulsions and the amount of dispersed phase. Asadpour et al. in 2016 showed that multiple nanoemulsions of folic acid had the highest stability at PH = 6. They also reported that the use of two surfactants, pectin and whey proteins, increases the stability of the prepared nanoemulsions (63).
It can be predicted that the confinement of food ingredients with multiple nanoemulsions will continue to grow with the further growth of these compounds in the food industry.

8. Conclusion

The encapsulation of bioactive food ingredients by nanoemulsion method has been significantly expanded in current years, and the main aim of scientists is to optimize this process for specific biomass. One of the main challenges in nanoemulsions is to increase the stability, which can be solved to a large extent by combining emulsifiers, controlling the production method of nanoemulsions and using biopolymers such as chitosan, etc. Another limitation of this encapsulation method is the inability to use both hydrophobic and hydrophilic materials in single nanoemulsions, the solution of which is the use of multiple nanoemulsions. Nanoemulsions are of special importance as enclosing compounds for food and pharmaceutical components.

Conflict of interest

The authors declare that they have no conflict of interest.

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