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Saffron Nanoemulsions: An Emphasis on the Two Recent Patents for Ultrasonic Nanoemulsion of Saffron

H. Ahari [a]***, S. Moradi*** [b]***, A. A. Anvar*** [c *]***, S. Allahyaribeik*** [d]

[a] Professor of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[b] MSc Student of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[c*] Associate Professor of the Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[d] Assistant Professor of the Department of Marine Sciences, Faculty of Natural Resources and Environment, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT: Nanoemulsions are a colloidal dispersion of oil/water or water/oil phases stabilized by emulsifiers. The physicochemical properties of nanoemulsions have made them suitable candidates for the delivery of bioactive compounds. Oil-in-water nanoemulsions are proper carriers for hydrophobic and water-in-oil or water-in-oil-in-water nanoemulsions are good carriers for hydrophilic compounds. Saffron is an expensive bulbous stemless herb whose different bioactive ingredients including crocins, crocetin, safranal, picrocrocin, and essential oils have valuable nutritional, medicinal, pharmaceutical, and antimicrobial properties. The use of nanoemulsion technology is a promising approach for saffron processing. The emulsion liquid membrane is one of the effective techniques for extracting saffron bioactive. On the other hand, encapsulating saffron extract in nanoemulsion droplets can increase the stability, bioavailability, and controlled release of bioactive ingredients of saffron. Due to its inherent antimicrobial activity, saffron can be used in the form of nanoemulsion coatings to increase the quality and shelf life of food products. In this study, the existing studies on saffron nanoemulsions processing were reviewed. The application of nanoemulsion techniques for saffron extraction, saffron bioactive nanoencapsulation, as well as the probable preservative properties of saffron essential oils for food packaging have been discussed. This article also reveals information on two recent patents on the subject of saffron nanoemulsion by ultrasonic method.

Keywords: Bioactive Compounds, Extraction, Encapsulation, Nanoemulsions, Preservatives, Saffron.

Introduction

Nanotechnology defined as the manipulation of particles with a size range of 1 to 100 nanometers (nm) has revolutionized the food industry. Nanotechnology has many potential applications in the food industry (Sekhon, 2010). It can be utilized in food production, processing, packaging, and storage as well as the improvement of the taste and color of food products. The use of nanoscale particles increases the surface-to-volume ratio that is improving the physical properties of food. Another aspect of the application of nanotechnology in the food industry is the use of nanoemulsions as carriers of lipophilic compounds (Chellaram *et al*., 2014; He & Hwang, 2016; Nile *et al*., 2020; Singh *et al*., 2017).

[*]Corresponding Author: Saaa4824@gmail.com

An emulsion is a colloidal dispersion of two immiscible liquids. Nanoemulsion is a colloidal dispersion of oil and aqueous phase (oil-in-water (o/w) or water-in-oil (w/o)) whose particle size is in the nano range. Apart from these two main phases, nanoemulsions consist of surfactants (emulsifier/stabilizer), cosurfactants, and additives. The oil phase of nanoemulsions can make up of various nonpolar components such as acylglycerols, free fatty acids, oil-soluble vitamins, and nutraceuticals (Anton & Vandamme, 2011; Singh *et al*., 2017). In the food industry, the use of triacylglycerol oils and nutritive oils such as soybean oil, castor oil, sesame oil, coconut oil, sunflower oil, olive oil, corn oil, and fish oils to produce emulsions is common. The aqueous phase of nanoemulsions predominantly is water. Surfactants are amphiphilic compounds added to nanoemulsions to improve their stability by reducing the surface tension. Ionic strength, pH, and long-term stability of the nanoemulsions are dependent on the surfactant used. Surfactants can be ionic (anionic or cationic), non-ionic or zwitterionic. Phospholipids, proteins, and polysaccharides are the most important emulsifiers that are vital for the stability optimization of nanoemulsion (Ashaolu, 2021; Azmi *et al*., 2019).

The globular structures of oil/water nanoemulsions are made up of an amphiphilic shell formed by surface-active compounds and a lipophilic core. Unlike emulsions that have a turbid appearance, nanoemulsions are transparent, thermodynamically unstable but kinetically stable. They are also resistant to gravitational separation and droplet aggregation. Therefore, they have good potential for encapsulating lipophilic nutrient compounds in delivery systems. Four important categories of functional compounds are encapsulated in nanoemulsions, which are: fatty acids, carotenoids, antioxidants, and phytosterols (Berton-Carabin *et al*., 2018; Hélder *et al*., 2012).

Nanoemulsion fabrication processes are divided into two general categories: high-energy and low-energy methods (Aswathanarayan & Vittal, 2019; Gharibzahedi & Jafari, 2018; Villalobos-Castillejos *et al*., 2018). In high-energy methods, two opposing forces named the droplet disruption force and the droplet coalescence force determine the droplet size of the nanoemulsion (Jafari *et al*., 2007). Only strong mechanical devices such as high-pressure valve homogenizers, ultrasonicators, and microfluidizers can produce intense disruptive forces and create small-scale oil droplets in the aqueous phase (Aswathanarayan & Vittal, 2019).

Nanoemulsions with a diameter of 1 nm can be prepared using high-pressure valve homogenizers. In these devices, the mixture of oil and water phases passes through a valve under the pressure of 500-5000 psi producing tiny emulsion droplets (Hélder Daniel Silva *et al*., 2012). The higher the pressure, the lower the interfacial tension and the faster the absorption of the material, the smaller particles are produced. This method is very efficient but consumes high energy and is extremely exothermic (McClements, 2011). In the ultrasonication method, the mixture of aqueous and oil phases is exposed to high-frequency sound waves (a frequency higher than 20 kHz) to transform large droplets into nanoemulsions (Jafari *et al*., 2007). In this method, the input energy, sonication time, emulsifier concentration, viscosity ratio of phases, and amplitude of the waves determine the droplet size (Leong, Wooster, Kentish, & Ashokkumar, 2009). The higher the frequency, the smaller the diameter of the nanoemulsions produced, therefore if frequencies in the MHz range are used, the emulsifier is no longer needed (Jafari *et al*., 2007). A noteworthy point in the ultrasonication method is that the nanoemulsions produced by this method can increase the antibacterial properties of the packaging material. Gahruie *et al*. examined the bioactivity of Zataria multiflora essential oil nanoemulsions incorporated in the basil seed gum-based film network. The results of this study showed that the antibacterial properties increase with the reduction of the size of nanoemulsion droplets formed by ultrasonic emulsification (Hashemi Gahruie *et al*., 2017). Within the microfluidizers devices, the mixture of the two phases, oil, and water, is forced to flow through an interaction chamber at high pressure of 500–20,000 psi. In microfluidization technique, although the temperature rises due to the use of high pressure, the obtained particles have a uniform size distribution (Mason *et al*., 2006).

The basic principle of the cost-effective low-energy methods is the use of physicochemical properties of the emulsion ingredients by applying a low amount of energy. There are various low-energy approaches utilized for the nanoemulsions production such as spontaneous emulsification, phase inversion temperature, phase inversion composition, membrane emulsification, solvent displacement, and emulsion inversion point method (Anton & Vandamme, 2009).

In the spontaneous method, the main factor for the formation of nanoemulsions is the physicochemical properties of the constituent compounds. Indeed, at a certain temperature, the oil phase, the aqueous phase, and the emulsifier are gently mixed resulting in the nanoemulsion droplet formation spontaneously (Solans & Solé, 2012). Compared to the high-energy techniques, the disadvantage of the spontaneous method is that the droplet size cannot be controlled. On the other hand, large amounts of synthetic surfactants are required for the stability of nanoemulsions (Date *et al*., 2010). But the simplicity and efficiency of this method have made it widely used in nanoemulsions of fat-soluble vitamins and fish oils (Aswathanarayan & Vittal, 2019).

Phase inversion temperature and phase inversion composition are two methods in which a water-in-oil emulsion is transformed to an oil-in-water emulsion using temperature changes and composition changes of the system, respectively (McClements, 2011). In the membrane emulsification method, a special membrane with the hydrophobicity/hydrophilicity property is used as an emulsifying agent. During the process, the dispersed phase is forced to pass through the pores of the membrane into the immiscible continuous phase (Oh *et al*., 2011). In the solvent displacement approach, which can be performed spontaneously at room temperature, first, the oil phase is mixed with the water-miscible organic solvent and then diffused into the aqueous phase. After the formation of nanoemulsion, the organic solvent is removed (Hélder Daniel Silva *et al*., 2012). The emulsion inversion point method can proceed when an emulsion of water/oil with a high oil-to-water ratio is converted into an emulsion of oil/water by reaching the catastrophic phase inversion point (McClements, 2011).

Different nanoemulsions have unique physicochemical properties that are significant in their application in the food industry. The optical properties of nanoemulsions, which depend on parameters such as particle composition, concentration, size, and distribution, are of great importance in the beverage industry. Nanoemulsions have their rheological properties and can improve the texture of various food products. Over time, nanoemulsions experience physical instability due to gravitational separation, droplet aggregation, Ostwald ripening, as well as chemical instability due to oxidation and hydrolysis. However, compared to conventional emulsions, they are more stable against aggregation and creaming due to having smaller particles (Z. Zhang & McClements, 2018).

Nanoemulsions can be used in various applications in the food industry. These applications include stability, bioavailability enhancement, antimicrobial, texture modification, flavor enhancement, nutrient enrichment, and colorant. Flavors and colorants are prone to oxidation due to their structural aldehydic, ketonic, or ester bonds. The use of nanoemulsions to encapsulate flavors and colorants increases their shelf life and improves their stability (Zhang *et al*., 2016). Saffron (*Crocus sativus L.*) is an herbal flavor and colorant that has great nutraceutical value due to the presence of bioactive compounds (Jafari *et al*., 2019). Saffron also has pharmaceutical effects and functions as an anti-carcinogenic, anti-inflammatory, and antidepressant agent (Moghaddam *et al*., 2018). The bioactive compounds of saffron can have a good function in the body when they are properly processed and delivered to the body while preserving their biological characteristics. So far, various techniques such as microencapsulation and nanoencapsulation have been used to preserve saffron bioactive ingredients. In recent years, nanoencapsulation has been considered a promising approach for encapsulating saffron bioactive compounds by surrounding them in preservative materials. This review article provides comprehensive information regarding the recent advances in saffron encapsulation techniques with an emphasis on nanoemulsions.

- ***Saffron (Crocus sativus L.)***

Saffron is an ancient bulbous stemless herb that is mostly cultivated in some specific countries, including Iran, India, Greece, Afghanistan, Morocco, Spain, Italy, and China with the worldwide production of 430, 22, 7.2, 6, 2.6, 2.3, 1, and 1 ton in 2019, respectively (www.statista.com/statistics/1135621/leading-saffron-producers-worldwide). Iran is the largest producer and exporter of saffron in terms of quantity and quality and contributes about 85-90% of the world’s saffron production. Most provinces are involved in saffron production in Iran, but Khorasan Razavi, South Khorasan, and North Khorasan have had the highest saffron cultivation areas (Milajerdi & Mahmoudi, 2014). Saffron grows in subtropical climates and sandy soils in autumn and is dormant during summer (Razak *et al*., 2017). During drying process, 80% of saffron moisture is lost and only 7 to 10% wt of it is preserved (Mokhtari & Pourabdollah, 2013). Different compartments of this plant contain a variety of compounds, including carotenoids, flavonoids, anthocyanins, vitamins (especially riboflavin and thiamine), minerals, and many other chemicals/biochemical compounds such as carbohydrates, proteins, and fats (Hosseini, Razavi, & Hosseinzadeh, 2018). Chemical composition analyses have shown that there are four major bioactive compounds in saffron including crocins, crocetin, picrocrocin, and safranal (Figure 1).

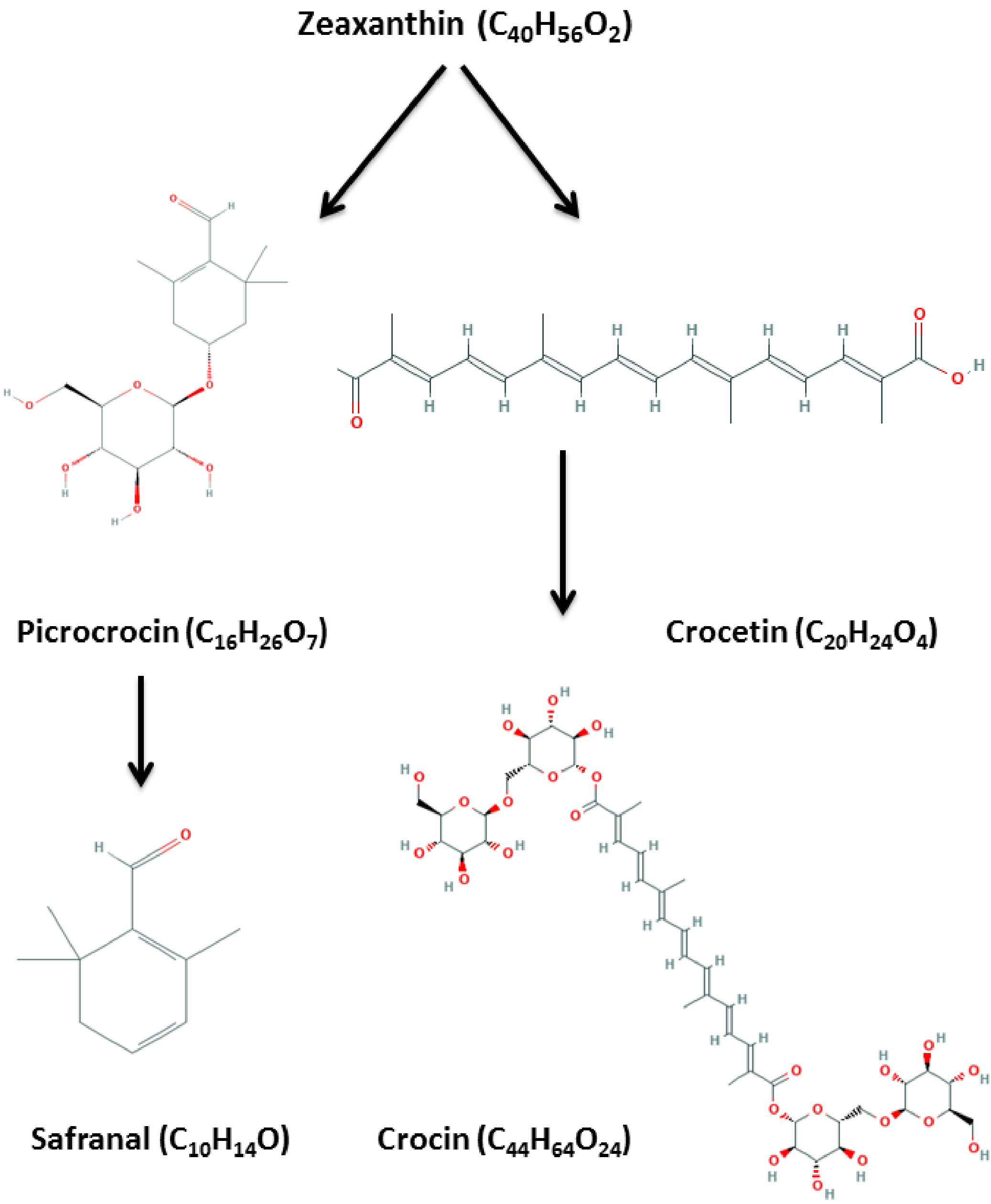

Fig. 1. Chemical structure of the major bioactive components of saffron. Bio-oxidative cleavage of zeaxanthin produces other compounds.

The color, aroma, and taste of saffron are related to the presence of these four bioactive components, which are more present in the stigma of the plant (Melnyk, Wang, & Marcone, 2010). Crocins in saffron are soluble in water (due to the presence of glycosyl groups) and form a golden red color. Crocins have antioxidant activity and exhibit therapeutic properties for diseases such as Schizophrenia and Alzheimer's. It has been shown that the antioxidant properties of saffron are even greater than those of carrots and tomatoes (Akhondzadeh Basti *et al.*, 2008). Crocin in a methanolic solution at a concentration of 500 ppm can inhibit up to 50% of free radicals. The high inhibitory activity of crocin is probably due to the reduction of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radicals (Assimopoulou *et al*., 2005). Crocetin is also a colorant and has medicinal, pharmaceutical, and nutritional properties. Picrocrocin, which causes the bitter taste of saffron, has anti-cancer effects. Safranal which is derived from picrocrocin, is the cause of saffron aroma and has anti-depressant properties (José Bagur *et al*., 2017).

In traditional Iranian medicine, saffron has been known for centuries as a food spice with strong medicinal properties. Saffron has been used as an antispasmodic, sedative, as well as menstrual regulative and abortifacient. This spice has beneficial medicinal activity on both central and peripheral nervous systems (Figure 2). A study by Ghaffari *et al*. showed that saffron extract can affect oxidative stress in the hippocampus and improve learning and memory impairment in an animal model of Multiple Sclerosis (MS) (Ghaffari *et al*., 2015). The crocins in saffron play a role in preventing or improving Alzheimer's disease and can be used as a medicine or supplement (Finley & Gao, 2017). In a study conducted by Ahmad and coworkers, on hemiparkinsonian male Wistar rats, pre-treatment with crocetin (25, 50, and 75 µg/kg body weight) for 7 days helped prevent Parkinsonism (Ahmad *et al*., 2005). The crocin in saffron may also be effective in preventing cerebral ischemia. The study of Vakili *et al*. indicated that crocin has the potential to protect a rat model of stroke against ischemia and cerebral edema (Vakili *et al*., 2014). Trans-sodium crocetinate (TSC) has also been used in clinical trial studies of cancer patients with resistant solid tumors and promising results have been obtained (Gainer *et al*., 2017). Saffron's positive effects on depression treatment and anxiety reduction are also frequently reported. A recent meta-analysis study showed that the effect of saffron was significantly higher than placebo on severe depression (Tóth *et al*., 2019). Many clinical trials have reported that the effect of saffron bioactive ingredients on depression and anxiety is similar to antidepressant medications (Shafiee *et al*., 2018). It can be concluded that saffron is a spice mostly used as a foodstuff, but its ingredients can have pharmaceutical applications in the treatment of various diseases.

- ***Nanoemulsion systems for extraction of saffron bioactives***

The emulsion liquid membrane (ELM) known as artificial kidneys in biomedical sciences is one of the effective techniques for extracting metal ions, organic acids, and biochemical compounds. This technique has many applications in the pharmacy, medicine, and food industries. The ELM method involves double emulsions (water-in-oil-in-water ($W_1/O/W_2$) including a homogeneous layer of an organic liquid (oil phase) that is placed between two aqueous phases. The inner aqueous phase (stripping phase W_1) contains the extractable and the outer (feed phase W_2) is the carrier phase enriched with the target compounds (Figure 3). The transfer of target molecules.

from the outer aqueous phase to the inner phase through the membrane is due to the concentration gradient (Kumar *et al*., 2019). A successful ELM extraction depends on various factors such as organic phase composition, surfactant composition, external phase compassion, external feed phase pH, internal phase composition, treat ratio, phase ratio, emulsification speed, and stirring speed, emulsification time, and temperature

(Abbassian & Kargari, 2016; Kumar *et al.*, 2019).

Inorder to extract saffron bioactives by ELM method, four steps need to be performed: one; preparation of water in oil emulsion (W_1/O), two; stirring the W_1/O emulsion on the feed phase (saffron suspension in water), three; separation of the external feed phase, and four; demulsification of W_1/O to get the enriched saffron bioactive solution. The second step is the main part of extraction during which saffron bioactive compounds transfer from the feed phase to the stripping phase. In 2013, Mokhtari *et al.* used the ELM method to extract saffron bioactive compounds (Mokhtari & Pourabdollah, 2013). This study showed that ELM system including Span 80 2.5 wt% as a surfactant, n-Decane as membrane, phase ratio 0.8 (4:5), and treat ratio 0.3 under stirring rate of 300 rpm can collect more than 90% of saffron biocompounds into the inner aqueous phase of designed.

In the extraction process, first, the saffron extract is in the continuous phase and then it is transferred to the internal phase. In the encapsulation process, the saffron extract is placed in the internal phase and is preserved by the membrane and continuous phases.

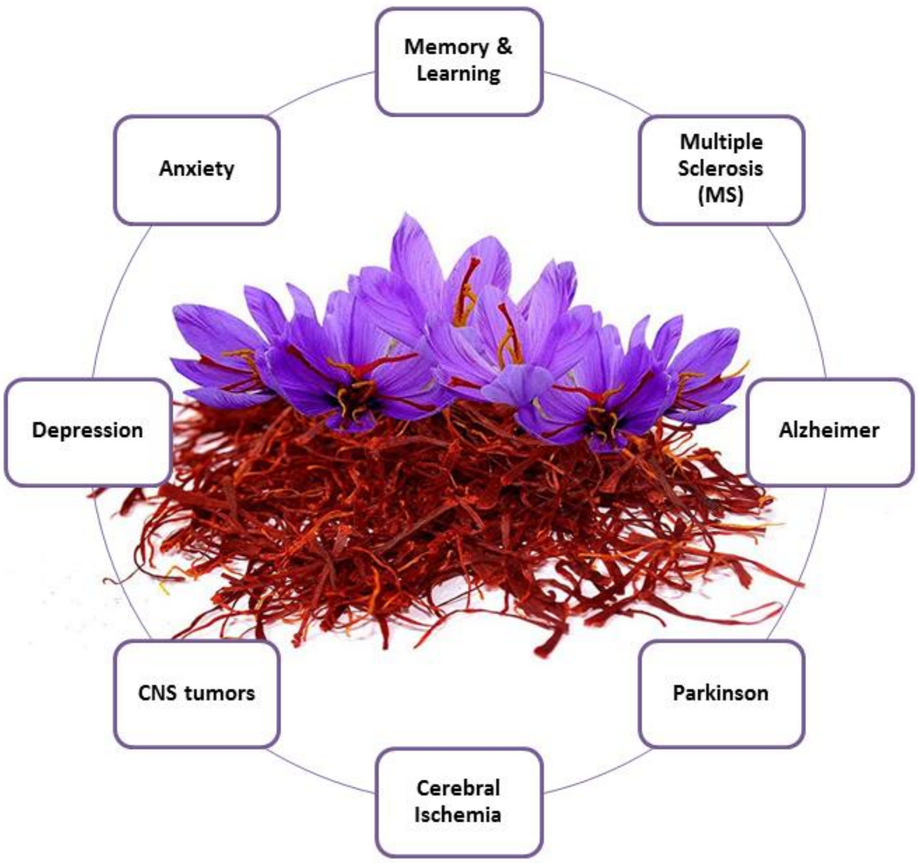

Fig. 2. Saffron has a pharmacological effect on both central and peripheral nervous systems.

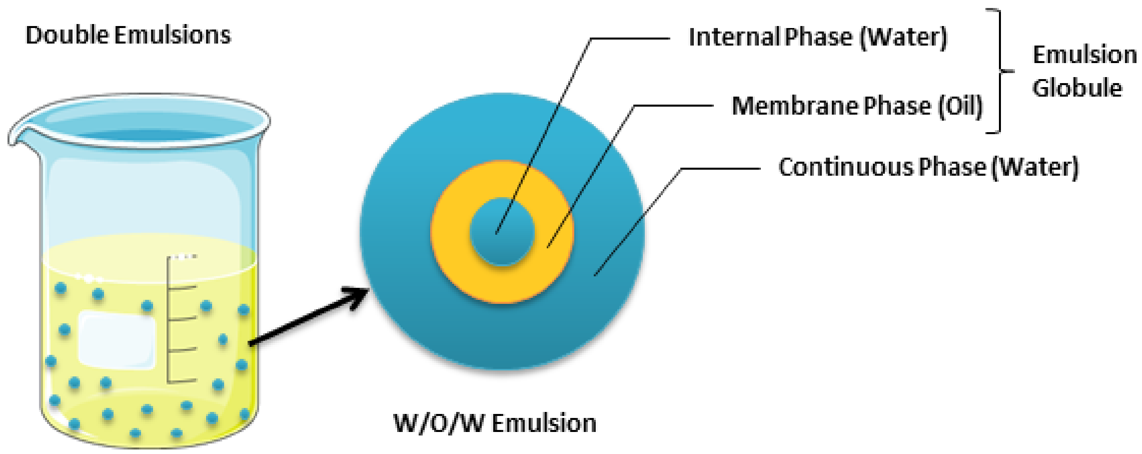

Fig. 3. Schematic drawing of the composition of double emulsions.

According to this study, phase ratio is the volume of the internal phase to the volume of the membrane, and treat ratio is the volume of the emulsion phase to the volume of the external phase (Mokhtari & Pourabdollah, 2013).

The emulsion diameter is very important for efficient extraction. The smaller the size of emulsions, the more stable they are. However, the excessive shrinkage of the size of the emulsion makes demulsification difficult to perform. Therefore, for efficient extraction, the formulation of the ELM system and concentration of different components are very important.

– ***Nanoemulsion systems for encapsulation of saffron bioactives***
– ***Nanoencapsulation of bioactive compounds***

Encapsulation technology is an approach of enclosing bioactive compounds into capsules with the aim of efficient delivery of them to the desired system. Nanoencapsulation that is a rapidly expanding branch of nanotechnology refers to the packaging of important functional bioactive ingredients at the nanoscale. The main purpose of nanoencapsulation is to protect bioactive against unfavorable environmental agents and deliver them to target organs in a controlled manner. Nanoencapsulation which provides particles of <100 nm has many benefits; it increases bioavailability, increases the stability of compounds, and protects them against oxidation, and enzymatic degradation (Ezhilarasi *et al*., 2013).

Principally, the structure of nanoencapsulation forms from a core covered by a wall. However, they can be organized into i) single-core, ii) multi-core, iii) single wall or iv) multi-wall. Phenolic compounds, carotenoids, essential fatty acids, vitamins, peptides, and enzymes are susceptible core compounds that can be nano encapsulated. Carbohydrates (chitosan, pectin, cellulose derivatives, modified starches, etc.), proteins (whey protein, soy proteins, gelatin, caseins, etc.), fat and waxes (hydrogenated vegetable oils, lecithin, bee wax, etc.), and polymers (polyethylene glycol, polyanhydrides, polyvinyl alcohol, etc.) are commonly used as wall materials for encapsulation of bioactive ingredients (Pateiro *et al*., 2021).

Many techniques have been introduced for nanoencapsulation of bioactive compounds. Jafari has classified these techniques into the following five categories; i) lipid-formulation-based techniques, ii) natural nanocarriers-based techniques, iii) specialized equipment-based techniques, iv) biopolymer nanoparticles-based techniques, and v) other nanoencapsulation technologies (Jafari, 2017). The first category is widely used in the pharmaceutical and food industries because most bioactive components and nutraceuticals have a hydrophobic lipid structure. Lipid-formulation-based techniques themselves are classified into several subgroups, which are: a) nanoliposomes, b) solid lipid nanoparticles, c) nanostructured lipid carriers, and d) nanoemulsions (Jafari, 2017).

Liposomes, which are made of at least one lipid bilayer especially phospholipids, have an aqueous core inside and are suitable for encapsulating hydrophilic compounds (Briuglia *et al*., 2015). Nanoliposomes are liposomes that are 50 to 150 nanometers in size. They can be used as a drug delivery spherical vesicle for hydrophilic nutraceuticals and pharmaceuticals (Jafari, 2017). Solid lipid nanoparticles are crystallized lipid droplets taking in the lipophilic bioactive components in their lipid matrix. They are a novel delivery system mostly utilized for pharmaceutical applications (Ghasemiyeh & Mohammadi-Samani, 2018). Nanostructured lipid carriers are unstructured-matrix droplets formed from the mixture of solid and liquid lipids. Since these nanocarriers contain liquid lipids in the inner phase, they have higher encapsulation efficiency than solid lipid nanoparticles (Beloqui *et al*., 2016). Nanoemulsions are one of the ideal vehicles for encapsulation, which we will discuss in detail in the next section.

- ***Nanoemulsion of bioactive compounds***

The most critical limitations of bioactive compounds are their low bioavailability and low stability. Low solubility, rapid metabolism, and volatility are the other restrictions of the bioactive food ingredients in food developing technologies (Aswathanarayan & Vittal, 2019). Encapsulation of bioactive compounds by the use of emulsion systems can overcome these limitations and supply the required physicochemical properties of bioactive during processing and storage conditions. Currently, a lot of research has been utilized the emulsification approach as a suitable way for encapsulation of various bioactive food compounds. In a study, Yang *et al* reported that citral, a flavoring compound used in food and cosmetics, became more stable by utilizing O/W nanoemulsions and the addition of the appropriate antioxidants (Yang *et al*., 2011). The positive effect of ubiquinol-10 ($Q_{10}H_2$) on increasing the chemical stability of citral in an O/W nanoemulsion system with the 1:1 ratio of $Q_{10}H_2$/citral has also been reported (Zhao *et al*., 2013). Another study showed that a nanoemulsion system containing the organic solution of beta-carotene (a natural colorant precursor of vitamin A with antioxidant properties) in the aqueous phase increase the stability of beta-carotene. In this study, Tan and Nakajima suggested that a special emulsifier system could improve the chemical stability of unstable beta-carotene (Tan & Nakajima, 2005). In the next study, they introduced polyglycerol esters of fatty acids (PGEs) as a nonionic emulsifier to improve physicochemical properties and stability of beta-carotene (Tan & Nakajima, 2005). According to Wei and Gao's study, the best physicochemical properties for beta-carotene nanoemulsions were obtained when a sodium caseinate-chitosan-epigallocatechin-3-gallate conjugates coating was used (Wei & Gao, 2016). In a study, tea polyphenols nanoemulsion was used to increase the bioavailability of beta-carotene. In this study, beta-carotene was in the oily phase of nanoemulsion and tea polyphenols were in the water phase. This nanoemulsion showed better stability, higher retention rate, and higher adsorption than beta-carotene nanoemulsion in both *in vitro* and *in vitro* studies (Meng *et al*., 2019). Edible vitamin D (cholecalciferol) nanoemulsions have been developed to enrich dairy products. Golfomitsou *et al* investigated edible O/W emulsions as vitamin D nanocarrier for the fortification of dairy emulsions. The used emulsifiers and the oil phase composition were polysorbate 20, soybean lecithin and their mixtures, and soybean oil or mixtures of the oil with cocoa butter, respectively. The vitamin D (0.1–0.5μg/mL) encapsulated in the oil core of nanocarriers (with mean diameters <200 nm) was added to whole-fat milk and remained stable for at least ten days (Golfomitsou, Mitsou, Xenakis, & Papadimitriou, 2018). According to Cheong *et al*., kenaf seed oil-in-water nanoemulsions containing emulsifiers of sodium caseinate, beta-cyclodextrin, and Tween 20 that encapsulate vitamin E and phytosterols can maintain the bioactive compounds stability and antioxidant activities up to 8 weeks in 4 ºC (Cheong *et al*., 2018).

Numerous studies have investigated the nanoemulsification approaches for the preservation of different functional bioactive compounds and their bioavailability, some of which have been mentioned (Table 1). Although the benefits of saffron, both as a food additive and as a substance with many medicinal properties, have been identified, the study of the use of nanoemulsions to encapsulate the active ingredients of saffron is limited. In the next section, the existing studies in this field are reviewed and summarized.

- ***Nanoemulsion of saffron bioactive compounds***

The active compounds in saffron have aldehyde, ketone, and ester functional groups which make them susceptible to oxidation reactions. Nanoencapsulation is a promising method for enclosing the bioactive compounds of saffron in different walls (Table 1). In their study, Garavand *et al*. divided saffron nanoencapsulation methods into five categories: i) nanoparticles, ii) nanostructured lipid dispersions, iii) nano-hydrogels, iv) electrospinning, v) nanoemulsions and nanodroplets (Garavand *et al*., 2019). Each of these methods has its physicochemical properties. In the following, we will review the nanoemulsion method in detail.

One of the informative studies in the field of saffron nanoencapsulation is the study of Esfanjani and colleagues. They encapsulated extract of saffron compounds (crocin, picrocrocin, and safranal) prepared according to Premkumar *et al*. method (Premkumar, Abraham, Santhiya, & Ramesh, 2003) in two model food systems of double or single layer W/O/W multiple emulsion systems. Maltodextrin-whey protein concentrate or maltodextrin-whey protein concentrate-pectin was used as wall materials. They also used sunflower oil and Span 80 for the oil phase. This study showed that double-layered W/O/W multiple emulsions stabilized by sequential adsorption of whey protein concentrate/pectin had a good performance to preserve the active compounds of saffron and its low yellow color surface (Esfanjani, Jafari, Assadpoor, & Mohammadi, 2015). In their next study, Esfanjani *et al*. showed that the

Table 1. Summary of studies used nanoemulsions for nanoencapsulation of plant bioactive compounds

Bioactive compounds	Wall material	Oil phase	Processing Technique	Effect	Reference
Citral	Soy lecithin	Medium-chain triacylglycerol/ undecane/$Q_{10}H_2$	High pressure homogenizer	With appropriate concentrations of $Q_{10}H_2$, major citral oxidation compounds can be inhibited to lower levels.	(Zhao *et al*., 2013)
Citral	Gelatin/ Tween 20	Medium chain triacylglycerl/ undecane	High pressure homogenizer	The mixture of gelatin and Tween 20 can enhance the citral nanoemulsions stability under acidic conditions.	(Tian *et al*., 2017)
β-Carotene	Starch	Medium chain triacylglycerl	High pressure homogenizer	Modified starches with lower film oxygen permeability have a higher retention of beta-carotene during storage.	(Liang, Huang, Ma, Shoemaker, & Zhong, 2013)
β-Carotene	Starch caseinate/chitosan-epigallocatechin-3-gallate	Sunflower oil	High pressure homogenizer	The produced bilayer nanoemulsions had better chemical stability, and dense and thick bilayer structure.	(Sheng *et al*., 2018)
β-Carotene	Casein	Vegetable oil	Ultrasonication and Microfluidization	Sonication-assisted dissolving methods together with freeze drying are effective to prepare β-carotene-enriched functional foods and dietary supplements.	(Chen, 2017)
Carotenoids extracted	Gelatin/ whey protein isolate	Soybean oil	High pressure homogenizer	Gelatin can increases water solubility of nanoemulsions of melon carotenoids extract	(Medeiros *et al*., 2019)
Crocin, picrocrocin and saffranal	Polysaccharide (maltodextrin) Protein (whey protein concentrate) Polysaccharide (pectin)	Sunflower oil Span 80	Spray drying	Efficient encapsulation of crocin, picrocrocin and saffranal due to stable wall materials	(Esfanjani *et al*., 2015)
Crocin, picrocrocin and saffranal	Polysaccharide (maltodextrin) Protein (whey protein concentrate) Polysaccharide (pectin)	Sunflower oil Span 80	Spray drying	A high stability and low release of encapsulated compounds up to 22 days	(Faridi Esfanjani *et al*., 2017)
Crocin	Polyglycerol polyrecioleate	Virgin olive oil Span 80	Spontaneous method	Polyglycerol polyrecioleate is a suitable surfactant for the preparation of saffron nanoemulsions.	(M. A. Mehrnia *et al*., 2016)
Saffron petal extract	Whey protein concentrate Basil seed gum Tween 80	Pure canola oil Vitamin D_3	High pressure homogenizer Ultrasonic homogenizer	Basil seed gum is a good emulsifier for improving the bioavailability of vitamin D_3 and saffron petal extracts.	(Gahruie *et al*., 2020)
Crocins and picrocrocin	Maltodextrin	---	Nano spray drying	Nanoencapsulation enhances the thermal stability and	(Kyriakoudi & Tsimidou, 2018)

Bioactive compounds	Wall material	Oil phase	Processing Technique	Effect	Reference
				bioaccessibility of saffron bioactives.	
Crocin	Polyglycerol polyrecioleate Whey protein concentrate Gum Arabic Angum gum	Virgin olive oil	Spontaneous method	The highest stability was seen when Angum gum was used in in outer aqueous phase of nanoemulsions.	(M.-A. Mehrnia, Jafari, Makhmal-Zadeh, & Maghsoudlou, 2017)
Crocin, picrocrocin and saffranal	Tween 80 Span 80	---	Ultrasonic homogenizer	The properties of W1/O emulsion have a great impact on the stability of the W1/O/W2 emulsion system and the preservation of saffron biocompounds.	(Najaf Najafi, Nemati, Mohammadi-Sani, & Kadkhodaee, 2020)
Curcumin	Sodium dodecyl sulphate	Medium chain triacylglycerl.	High pressure homogenizer	Multilayer nanoemulsions had a lower curcumin bioaccessibility than uncoated nanoemulsions	(Hélder D. Silva *et al*., 2018)
Curcumin	Tween 80 Lecithin Whey protein isolate Acacia	Canola oil Linseed oil Medium-chain triglyceride	High pressure homogenizer	Tween 80 with higher surfactant-to-oil ratio values produces more stable curcumin nanoemulsions	(Ma *et al*., 2018)
Curcumin	Tween 80 Glycerol	Cinnamon essential oil Garlic essential oil Sunflower oil	Spontaneous emulsification	Chilled chicken fillets coated with curcumin nanoemulsions make had higher sensory scores	(Abdou, Galhoum, & Mohamed, 2018)
Vitamin D	Polysorbate 20 Soybean lecithin	Soybean oil Cocoa butter	High pressure homogenizer	Since the concentration of vitamin D affects the size of the oil cores, the concentration should be such that the stability of the nanoemulsions is maintained.	(Golfomitsou *et al*., 2018)
Vitamin D	Pea protein	Canola oil	High pressure homogenizer	Pea protein is an effective emulsifier suitable for vitamin D nanoemulsions.	(Walia & Chen, 2020)
Vitamin D	Pea protein	Canola oil	Ultrasonic homogenizer	The pea protein nanoemulsions can be served as the potential carrier and stabilizer of vitamin D.	(Akkam *et al*., 2021)
Vitamin D	Tween 80	Cinnamon oil	Ultrasonic homogenizer	Cinnamon oil is a good carrier of vitamin D.	(Meghani *et al*., 2018)

designed W/O/W multiple emulsion system (maltodextrin-whey protein concentrate-pectin) can preserve the active compounds of saffron up to 22 days. These systems have a low release of encapsulated bioactive and provide high protection of them in gastrointestinal conditions (Faridi Esfanjani, Jafari, & Assadpour, 2017). Due to a controlled release of bioactive ingredients in the body, pectin is a promising wall material for nanoemulsion of saffron bioactive. The use of pectin along with proteins, lipids, and other polysaccharides enhances its beneficial effects (Rehman *et al*., 2019). In another study, Mehrnia *et al*. showed that spontaneous emulsification as a low energy method and polyglycerol polyrecioleate and Span 80 as nonionic surfactants have good potential for producing stable crocin nanoemulsions (Mehrnia *et al*., 2016). In a study conducted by Gahruie *et al*., saffron petal extract co-encapsulated with vitamin D_3 in nanoemulsions with different emulsifiers (whey protein concentrate, basil seed gum, and Tween 80). This study introduced basil seed gum as a good stabilizer for emulsifying vitamin D_3 and saffron petal extracts in food nanoemulsions (Gahruie *et al*., 2020). It has also been reported that the ratio of core to the wall is a critical factor in the encapsulation efficiency of saffron extract. Kyriakoudi *et al*. encapsulated saffron extract in maltodextrin using the spray drying method. They used caffeic acid as a strong phenolic antioxidant in the feed phase of nanoemulsions to test their stability under thermal and gastrointestinal conditions. The results showed that the ratio of core to the wall has an important effect on the efficiency of nanocapsules and caffeic acid increases their stability under both thermal and gastrointestinal conditions (Kyriakoudi & Tsimidou, 2018).

Recently, a new nanoemulsion formulation of saffron extract and a method of preparation thereof which is patented in the Patent Application Publication of the United States (Pub. No.: US 2021/0046141 A1, Pub. Date: Feb. 18, 2021) have been disclosed. In the formulation, saffron stigmas were first crushed using liquid nitrogen. Then the extract was obtained by solving the crushed saffron in a non-polar solvent (n-decane) using the ultrasonic method. Polyoxyethylene (20) sorbitan monolaurate, glycerol, and maltodextrin were used as surfactants in the aqueous phase. Sorbitan monooleate was added to the extract for obtaining an oil phase. Oil-in-water nanoemulsions (particle size of 23 nm) were obtained by a high-speed homogenizer followed by an ultrasonic homogenizer. According to the inventors, this formulation causes a more intense color, richer and stronger odor, more suitable aroma, and better taste and increases the shelf life of saffron extract.

In another patent entitled water-in-oil nano-emulsion of saffron and a method of preparing thereof (Pub. No.: US 2021/0161987 A1, Pub. Date: Jun. 3, 2021), saffron stigmas were first crushed in liquid nitrogen, and then the extract was prepared in aqueous solvent using the ultrasonic method. A high-speed homogenizer followed by an ultrasonic homogenizer was performed to obtain a water-in-oil nano-emulsion. The surfactant used in the oil phase (olive oil) was sorbitan monooleate (44.5%) and the surfactant used in the aqueous phase was polyoxyethylene (20) sorbitan monolaurate (2.5%). According to the inventors of this method, it is an economical approach that requires small amounts of saffron and at the same time improves the physical properties (color, odor, and flavor) as well as the shelf life of the extract.

Saffron nanoencapsulation technology gives new opportunities to food and pharmaceutical industries to preserve its color, taste, and aroma, as well as bioactive compounds in both environmental and gastrointestinal conditions. However, due to the limited number of current studies, further investigations and collaboration of research laboratories with industry are needed.

- ***Saffron Nanoemulsions as natural preservatives***

One of the most important reasons for food spoilage is the presence of microorganisms. Microbial activity in food is very worrying because it endangers the health of the consumer and causes great economic losses. Using safe preservatives is one way to overcome microbial spoilage in food products. Essential oils of some plants have excellent antimicrobial activity against food microorganisms, however, they are hydrophobic and it is difficult to apply them in food packaging industries directly (Bondi *et al*., 2017). Nanoemulsions can help to solve this problem by keeping the antimicrobial oily compounds of plants in the oil phase of nanodroplets. Recently, some studies have been performed on nanoemulsions produced from plants oils to increase the shelf life of food products. For example, in a study conducted by Nasiri *et al*., the potency of nanoemulsions containing the essential oil of three different plants (*Rosmarinus officinalis L*., *Zataria multiflora Boiss*, and *Cuminum cyminum L.*) to increase the *Acipenser stellatus* filet shelf life were examined. The results showed that *Cuminum cyminum L.* nanoemulsion produced by ultrasonic homogenization had the best antimicrobial effect on fish samples (Nasiri *et al*., 2020). Another study has been confirmed the effect of *Polylophium involucratum* nanoemulsions on decreasing the microbial count and improving the storage time of *Green Tiger Pawn* (Bahrami, 2019). The ability of curcumin essential oil nanoemulsions produced by emulsion phase inversion to the extension of the shelf life of *Oncorhynchus mykiss* has also been reported (Lahidjani *et al*., 2020).

Saffron, like many other plants such as orange, clove, and thyme, has bioactive ingredients that have antimicrobial properties (Aswathanarayan & Vittal, 2019). Due to this inherent antimicrobial property, saffron is considered a natural preservative to improve the shelf life of foods. Saffron is resistant to pathogens such as *Salmonella* and, unlike other spices, is not degraded. Pintado *et al*. evaluated the antimicrobial properties of saffron and showed that the bioactive ingredients of saffron, especially safranal (8–16 mg/mL), and crocin (64–128 mg/mL) can significantly reduce the risk of saffron contamination with *Salmonella* (Pintado, 2011). Other studies have shown the antimicrobial properties of saffron against bacteria including *Microccucos luteus, Staphylococcus epidermitis, Staphylococcus aureus, Escherichia coli, Brucella*, and fungi including *Candida albicans, Aspergillus niger,* and *Cladospourium sp* (Hosseinzadeh & Nassiri-Asl, 2013; Motamedi *et al*., 2010).

Due to the antimicrobial properties of saffron, it is possible to use saffron nanoemulsions as a safe natural preservative in food packaging. Recently, a study investigated the effect of saffron nanoemulsion on shrimp shelf life using two methods of spontaneous emulsion and ultrasonic homogenization. This study showed that 5% nanoemulsion of saffron by homogenizer method has good antimicrobial effects against shrimp spoilage (Aboutorab *et al*., 2021). Since saffron has unique nutritional and medicinal properties in addition to its antimicrobial activity, more extensive studies are needed to use this colorful spice as a food preservative.

Conclusion

Nanoemulsions are colloidal droplets produced by mixing one immiscible liquid with another. The various physicochemical properties of nanoemulsions have made them prone to widespread use in the food industry. Saffron is the most valuable additive that in addition to its good color and flavor has unique nutritional, medicinal, and antimicrobial properties. Various studies have investigated the application of nanoemulsions in saffron processing. These studies can be divided into three areas: extraction, bioactive compounds encapsulation, and application in increasing food shelf life. In the present study, we reviewed the literature on these three areas and summarized their information. The use of nanoemulsions to extract the bioactive compounds of saffron allows the extraction of hydrophilic and hydrophobic compounds in different fractions. Saffron nanoencapsulation is an effective approach to increase the bioactivity and bioavailability of its bioactive compounds and can also increase their storage stability due to the size of nanoparticles. The use of infeed model food systems is very promising and has a significant influence on the preservation of encapsulated saffron extract. Saffron nanoemulsions also have potential applications in increasing the shelf life of foods. Despite the different capabilities of saffron nanoemulsions, studies have been limited and there is no unique method for use on industrial and commercial scales in any of the three areas. Many studies are needed to provide practical techniques for extracting, encapsulating, and using saffron as an antimicrobial.

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