Recent progress in oil-in-water-in-oil (O/W/O) double emulsions

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Abstract

Oil-in-water-in-oil (O/W/O) double emulsions are recognized as an advanced design route for oil structuring that shows promising applications in the pharmaceutical, cosmetic, and food fields. This review summarizes the main research advances of O/W/O double emulsions over the past two decades. It mainly focuses on understanding the preparation strategies, stabilization mechanism, and potential applications of O/W/O double emulsions. Several emulsification strategies are discussed, including traditional two-step emulsification method, phase-inversion approach, membrane emulsification, and microfluidic emulsification. Further, the role of interfacial stabilizers and viscosity in the stability of O/W/O double emulsions will be discussed with a focus on synthetic emulsifiers, natural biopolymer sand solid particles for achieving this purpose. Additionally, analytical methods for evaluating the stability of O/W/O double emulsions, such as advanced microscopy, rheology, and labelling assay are reviewed taking into account potential limitations of these characterization techniques. Moreover, possible innovative food applications are highlighted, such as simulating fat substitutes to decrease the trans- or saturated fatty acid content and developing novel delivery and encapsulation systems. This review paves a solid way for the exploration of O/W/O double emulsions towards large-scale implementation within the food industry.

Keywords: O/W/O double emulsions; emulsification; stabilization mechanism; innovative food.

1. Introduction

A double emulsion can be considered as one special emulsion of an emulsion, of which the configuration is that droplets of one dispersed liquid are further dispersed in another liquid. The intermediate phase separates the inner dispersed droplets from the outer phase. The two major types of double emulsions are water-in-oil-water (W/O/W) and oil-in-water-in-oil (O/W/O) emulsions as shown in Figure 1a and 1b(Muschiolik & Dickinson, 2017). Different combinations of the inner oil-in-water (O/W) interface (Figure 1c) and outer water-in-oil (W/O) interface (Figure 1d) allow the construction of O/W/O double emulsion systems with various structures. Due to their complex structure and unique nature, the separated phases of double emulsions can be constructed in such a way to regulate the incorporation of biologically active compounds and nutritional substances. In light of these facts, they show tremendous potential for applications in food, pharmaceutical, and cosmetic fields(Huynh Mai, Thanh Diep, Le, & Nguyen, 2019).

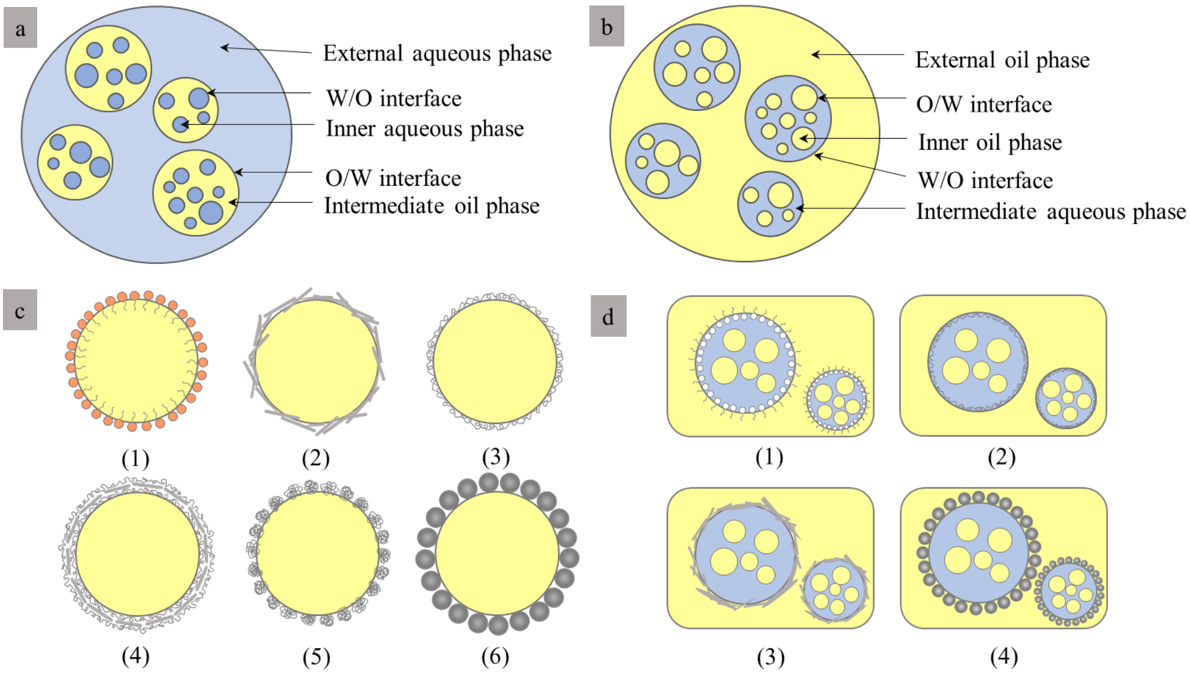

Figure 1 Main types of double emulsions. a. O/W/O emulsions; b. W/O/W emulsions; c. Different types of primary O/W interface: (1) low-Mw hydrophilic surfactants, (2) fibrils, (3) polymers, (4) multilayers, (5) aggregates, (6) solid particles; d. Different types of outer W/O interface: (1) low-Mw hydrophobic surfactants, (2) polymers, (3) crystals, (4) solid particles.

In contrast to W/O/W emulsions, less research focuses on O/W/O emulsions in the past decades. Thus, exploration of O/W/O systems are highly desirable, the reason of which could be as follows. First, healthy products can be fabricated by replacing the traditional W/O emulsion with the equivalent O/W/O emulsions, having a lower content of saturated fatty acid while be of a similar in-mouth perceived texture(Dickinson, 2010a). Second, O/W/O emulsions enable sensitive oil-soluble ingredients (such as essential oils and bioactive components) being protected and encapsulated, and then in turn control the release or delivery of these ingredients during consumption and/or digestion. Third, O/W/O emulsions could act as internal reservoirs to restore matters converted from the external continuous phase into the internal restricted confined phase, aiming to remove the toxic or unhealthy matters(Muschiolik & Dickinson, 2017).

Thus, the formulation of a stable O/W/O structure with food-grade ingredients is highly desirable by food researchers. However, the O/W/O structure is generally thermodynamic unstable and is prone to rupture during storage or when exposed to environmental stress. Noteworthy, the difference between the inner and outer phases will make the as-formed O/W/O emulsion being unstable, like sedimentation, flocculation, coalescence, and Ostwald ripening between droplets. It suggests that the O/W/O system should require an additional stability parameter to tailor the phase change(Leister & Karbstein, 2020). To gain further insight of the O/W/O emulsion as well as for their controllable large-scale fabrication, we prepare this review and aim to summarize the preparation methods, the stability mechanisms, and their applications.

2. Preparation of the O/W/O emulsion

An O/W/O emulsion includes two distinctive interfaces that require two different emulsifiers. The internal O_1/W interface should be stabilized by a hydrophilic emulsifier while the external interface requires a hydrophobic one, which finally forms the O_1/W/O_2 emulsion. Generally, four approaches for preparing the O/W/O emulsion can be considered: traditional two-step emulsification method, one-step homogenization, membrane emulsification, and microfluidic emulsification. Figure 2 shows a schematic diagram of these feasible methods for preparing O/W/O emulsions.

Among these strategies for fabrication of O/W/O emulsion, the most commonly used one is the traditional so-called ‘two-step’ approach as shown in Figure 2a. At first, a high energic treatment obtained with intensive shearing homogenizer(Triplett & Rathman, 2008), microfluidizer(Kadian, Kumar, Badgujar, & Sehrawat, 2021; Verma, et al., 2021), ultrasound(Xiong, et al., 2019; Y. Zhang, et al., 2018; Zhou, Zhang, Xing, & Zhang, 2021), and high-pressure homogenizer(Bi, et al., 2020; Jiang, Zhang, Zhang, & Peng, 2021) are utilized towards preparation of stable primary O/W emulsions with hydrophilic feature. After that, the primary emulsion is mixed with the external oil (containing the hydrophobic emulsifier), and then they are subjected to a further stirring treatment, which in turn finally forms O/W/O emulsion. Cho and Park(Y.H. Cho, 2003) prepared a primary O/W emulsion using a microfluidizer at 41 to 82 MPa, in which a maltodextrin/gum arabic mixture was serving as the wall material. The primary emulsion was re-emulsified at 13500 rpm for 10 min during a heating treatment in molten hydrogenated palm kernel oil containing 5% emulsifier, after which the final product of an O/W/O emulsion was formed upon cooling. Different from the one that was solely prepared with pure polyglycerin polylysinoleate (PGPR), it was found that the mixture of PGPR and sorbitan monooleate (Span 80) resulted in a narrower size distribution of outer droplets in the O/W/O emulsion. In order to meet the consumers' demand of free-synthetic surfactants and achieve the clean-label goal, Patel(Ashok R. Patel, 2017) successfully prepared O/W/O emulsions solely stabilized by natural polysaccharides and plant fat crystals with a two-step emulsification approach. The primary O/W emulsion was formulated by dispersing oil into a gelatin solution and then subjected to an ultra-turrax at 11000 rpm. Next, a xanthan gum solution was added in under continuous stirring. The as-prepared O/W emulsion was homogenized with molten palm oil containing different proportions of sunflower oil, which was then stable at 5 ^{o}C. These results suggested that a stable double emulsion could still be achieved without any involvement of emulsifier when the water content was up to 45 wt% and the corresponding inner oil phase down to 15 wt%.

It is worth noting that the methodological selection in the 2nd emulsification step (dispersing O_1/W into O_2) plays a critical role in the formulation of well-structured multiple emulsion system. On the other hand, it was noticed that the primary emulsion is prone to be unstable in the 2nd step, in case it is subjected to high energetic treatment, such as high-shear mixers, high-pressure homogenizers and ultrasound, the reason of which should be due to the extremely intense hydrodynamic force. To address this issue, suitable methods should be utilized. So far, numerous strategies, such as reducing the O_1 droplet size, improving the viscosity of the O_1 phase or the water phase, structuring interfacial complexes to improve the stability of inner emulsions, and incorporating surfactants, polymers, and colloidal particles in the outer oil-soluble phase, have been developed(Muschiolik & Dickinson, 2017).

Besides, one-step phase inversion was also reported to be a feasible strategy for the formulation of O/W/O emulsions, the mechanism of which was suggested to be attributed to the diffusion of oil from the continuous external phase to the inner phase (Figure 2b). Using this one-step method, Oh et al.(Oh, Park, Shin, & Oh, 2004) fabricated one kind of O/W/O emulsion system, in which Span 80 was working as a lipophilic emulsifier toward formulation of the W/O emulsion. When Tween 20 was added into the water phase, the stable W/O interface would be out of balance, in turn accelerating the diffusion of the oil phase (1-octanol) into water droplets. It was also found that using a polymer in the emulsion system could restrain the transfer of inner liposoluble substances into the other phase and the swelling of W/O interfaces. However, when adding polyethylene glycol (PEG) and hydroxypropyl cellulose (HPC) into the continuous oil phase and discontinuous phase, respectively, the micelles (bound to oil molecules) would be promoted to diffuse into the core of the aqueous phase. In light of these facts, it was suggested that the involvement of PEG and HPC in the corresponding phase favoured the formulation of the O/W/O emulsion. Klahn et al.(J.K. Klahn, 2002) claimed that phase inversion was governed by the interplay between droplet coalescence and the escape of the internal droplets; in other words, O/W emulsions would experience catastrophic phase inversion at appropriate shear rates. Thus, we can conclude that the phase inversion could bring about the formation of nano- and fine O/W/O double emulsions.

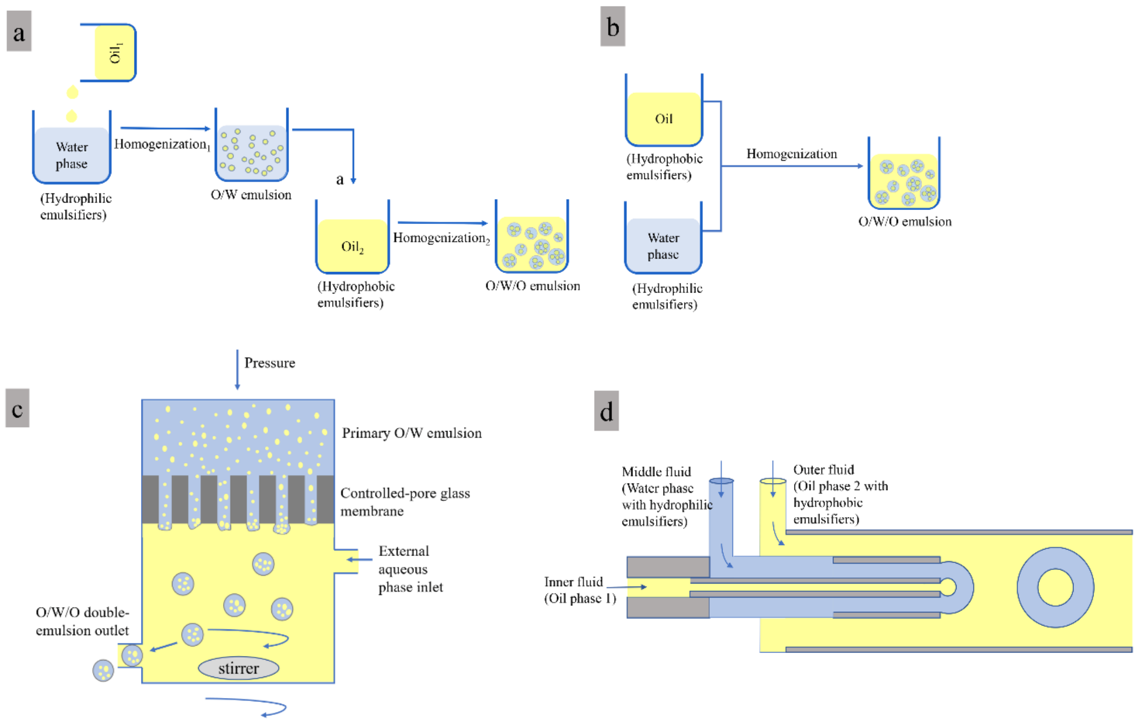

Figure 2 Popular methods for preparing O/W/O emulsions. a. Traditional two- step approach; b. One-step catastrophic phase inversion; c. Membrane emulsification; d. Microfluidization.

The membrane emulsification technique is acknowledged as another smart strategy for producing O/W/O emulsions(Akamatsu, Kurita, Sato, & Nakao, 2019) (Figure 2c). Before passing through the controlled micropore glass membrane, the primary O/W emulsions is generally fabricated via high-pressure homogenizer, sonication, microfluidizer as well as other homogenization methods. During the formation process, the primary emulsion is supplied to the upper chamber of the reactor, and an external oil phase is continuously placed into the counterpart lower chamber. Meanwhile, nitrogen is delivered into the upper chamber for providing a driving force, forcing the primary O/W emulsion passing through the controlled-pore glass membrane into the lower chamber, which generates the final O/W/O emulsion. Interestingly, the mean droplet size of the multiple emulsions can be precisely controlled over a wide range by changing the membrane pore size or varying the stirring speed. However, few studies report this technology to prepare O/W/O double emulsions, thus its applications need to be further investigated.

A new advanced approach for formulating the O/W/O emulsion was developed, i.e. microfluidic homogenization, which is a modified microchannel emulsification method(Khalid, Kobayashi, Neves, Uemura, & Nakajima, 2018). Figure 2d exhibits a schematic diagram for preparation of an O/W/O emulsion by the microfluidic homogenization approach. It shows that oil droplets with uniform size are injected into the middle aqueous phase to form the O/W emulsion via a capillary microfluidic device, after which the primary emulsion flows into the outer oil phase and subsequently the double emulsion is formed. The size and breakup rate of the droplets are determined by the flow rate in the chambers. The amount of microencapsulated aqueous droplets also can be tailored by altering the breakup rates at the junctions. With all these merits, we anticipate that this approach could be well utilized for preparation of monodisperse or multi-disperse double emulsions with highly controlled droplet size(Fang, Cao, Yu, & Li, 2019; Hwang, Oh, & Oh, 2005). Therefore, the advantage of this technique is that it can provide a higher degree of control over the double emulsion properties. However, it is not yet applicable to large-scale production.

3. Stabilization of the O/W/O emulsion

3.1 Instability mechanism of the O/W/O emulsion

The thermodynamic instability behaviours of traditional O/W and W/O emulsions are mainly shown as follows:

- Flocculation: Several droplets tend to move towards each other and then aggregate. Flocculation can lead to an increase in the effective particle size, therefore accelerating the creaming rate. Besides, it can accelerate the coalescence activities, resulting in the destabilization of the emulsion(Dickinson, 2019).

- Coalescence: After flocculation occurs, droplets irreversibly merge together to form larger droplets that will eventually be separated from the external phase. The driving force for emulsion coalescence is disruption of the liquid film between the droplets as well as making it thinner(Yarranton, Urrutia, & Sztukowski, 2007).
- Sedimentation or creaming: Gravity affects the migration of the dispersed phase of an emulsion, making resulting in sedimentation or creaming. Particle movement depends on the droplet size and the density difference between the dispersed and continuous phases, as well as viscosity and thixotropy(Loi, Eyres, & Birch, 2019; Zembyla, Murray, & Sarkar, 2020).
- Ostwald ripening: Small droplets with a large curvature dissolve faster than the large droplets with a small curvature. Thus, the small droplets tend to diffuse and finally deposit on the interface of larger droplets. The generation of large droplets is ultimately achieved at the expense of small droplets(Han, Song, Moon, & Choi, 2018). The driving force for Ostwald ripening activity is the difference in solubility between dispersed emulsion droplets(Zeeb, Gibis, Fischer, & Weiss, 2012).

Compared to traditional emulsions, double emulsions are thermodynamically more unstable because of their complex architecture(Aguiar, das Gracas Fernandes da Silva, Fernandes, & Forim, 2020). The O/W/O system is divided into different phases by two interfaces, so instability phenomena could occur in both internal and external emulsions (shown in Figure 3). Generally, the first instability is that the hydrophilic emulsifier and the hydrophobic emulsifier cannot be completely adsorbed on the corresponding interface, resulting in flocculation, coalescence, creaming, Ostwald ripening, and sedimentation between the small inner oil droplets within the water phase, as well as water droplets within outer oil phase(Choi, Kim, Cho, Hwang, & Kim, 2011). Moreover, the second one is that the inner oil phase favours to diffuse and migrate to the external interface due to the osmotic pressure difference (Yang, et al., 2021) or reverse micellar transport effect(Jochen Weiss, 1996). The coalescence of the smaller inner droplets with the interface of the outer droplets would occur, resulting from the thin nonaqueous film formed between the outer continuous phase and the internal oil droplets. Afterwards, this phenomenon irreversibly converts O/W/O emulsions into W/O emulsions with a simple structure, which enables the release of inner lipid-soluble substances successfully(Heidari, Jafari, Ziaiifar, & Malekjani, 2021). The former instability feature results in enlarged average size of the droplets, while the latter causes a complete delivery of the small inner droplets toward the outer phase. Other scientists also suggested that the main reasons for emulsion instability are: 1) the aggregation of the inner and double-emulsion droplets; 2) rupture of the intermediate phase on the surface of the inner droplets; 3) seepage of encapsulated complement from the inner to the outer phase; 4) shrinkage and swelling of the inner droplets in the O/W/O double emulsion because of osmotic gradients across the oil-liquid film; 5) phase separation(Dickinson, 2010a). Nonetheless, it should be pointed out the difference between different mechanisms is small, and even some of them are essentially similar. Also, the stability of the O/W/O emulsion depends on various factors, i.e. the types of emulsifiers, viscosity, environmental stress, and structural features, which cannot be explained by a simple proposed mechanism(Aserin, 2008).

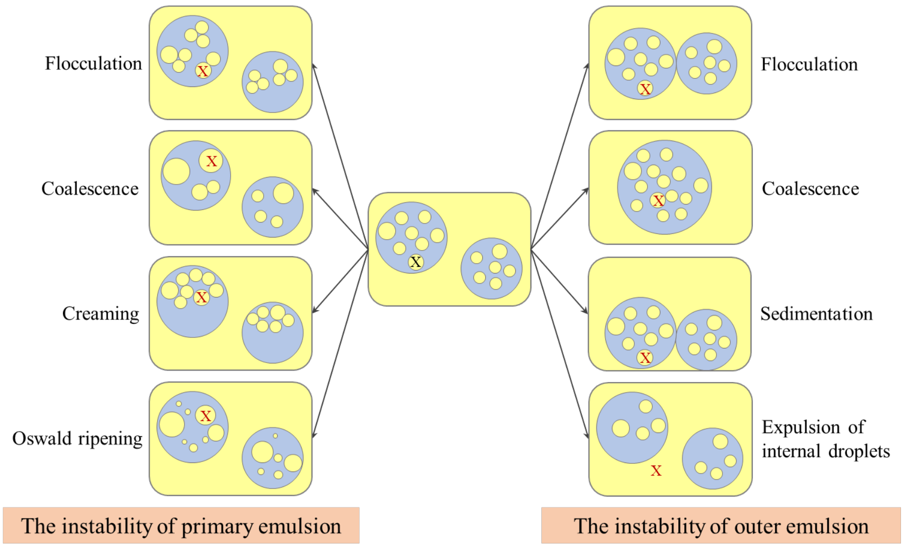

Figure 3 Schematic representation of instability pathways in the O/W/O emulsion.

3.2 Stabilization by macromolecules

Different emulsifiers have been utilized to formulate O/W/O emulsions, including synthetic surfactants and biopolymers. Numerous reports have shown that the types of the emulsifiers directly affect the properties of the interfaces, further altering the stability of emulsions(M. Li, McClements, Liu, & Liu, 2020; Zembyla, et al., 2020). Therefore, the selection of interfacial stabilizers is crucial for the stability of the double emulsion. For low-molecular-weight surfactants (i.e. monomeric emulsifiers, such as Span, methyl methacrylate), it is impossible to capture the inner phase for long time storage, the reason of which should be attributed to the fact that the monomeric emulsifiers are inclined to migrate from the inner and outer interfaces to the intermediate phase where they aggregate to generate micelles(Pratap, Datir, Mane, & Shukla, 2021). To enhance the stability of the emulsion, polymeric surfactants (such as proteins, polysaccharides, and their mixtures) can be chosen to substitute the monomeric ones, thus improving the degree of interfacial coverage during emulsification, by which better encapsulation and controlled released of the oil was achieved(Ding, Serra, Vandamme, Yu, & Anton, 2019). Since high concentrations of synthetic surfactants, such as Tween and Span, are required to further improve the stability and prolong the shelf life, their wide application in food and drug fields are greatly hindered(Muschiolik & Dickinson, 2017; Weiss, Scherze, & Muschiolik, 2005). Of note, natural macromolecular stabilizers are recognized as a "Clean Label" in food and pharmaceutic O/W/O systems(A. Benichou, Aserin, & Garti, 2004; Axel Benichou, Aserin, & Garti, 2007; Dickinson, 2010a; Muschiolik & Dickinson, 2017; Thanh Diep, et al., 2018). Compared with low-molecular-weight emulsifiers, the macromolecules adsorbed at the surface can improve the coverage, thus increasing the emulsion stability. The stabilization feature can be accessed by (1) depletion stabilization caused by unabsorbed polymers that inhibit particle collisions and increase the viscosity of the system; (2) electrostatic repulsion between two droplets with the same type of charge; (3) steric repulsion due to hydrophobic interactions between adsorbed polymers. However, macromolecular emulsifiers, such as proteins, polysaccharides and their complexes, are unlikely to completely substitute lipophilic monomeric surfactants in the double emulsion(A. Benichou, et al., 2004).

In recent studies, the biopolymers were applied as emulsifiers in food and drug multiple emulsions. Liao et al.(Liao, Luo, Zhao, & Wang, 2012) designed an O/W/O emulsion using modified succinic acid deamidated wheat gluten (SDWG) microspheres to encapsulate fish oil. By optimizing the SDWG concentration and the ratio of fish oil to SDWG, high encapsulation efficiency (EE) of 81.8% could be achieved, in which SDWG could improve the storage stability and release property. Diep et al.(Thanh Diep, et al., 2018) combined sodium caseinate (SC), k-carrageenan (KC) with soy lecithin to stabilize the O/W/O emulsion via a developed one-step emulsification method and found that SC played an emulsifying role while KC served as a emulsifier to inhibit the sedimentation within the aqueous phase. Qin et al.(Qin, Li, & Hu, 2021) developed a composite shell composed of biodegradable chitosan and gelatin to encapsulate n-tetradecanol for the production of the primary O/W emulsion, followed by emulsifying with liquid paraffin to formulate the O/W/O emulsion, and then crosslinked with glutaraldehyde (GTA). The result clarified that the formulated microcapsules possessed great thermal stability. Also, Benichou et al.(Axel Benichou, et al., 2007) reported the mixture of whey protein isolates (WPI) and xanthan gum hybrid (XGH) could significantly enhance the stability of primary O/W emulsion droplets, leading to a high encapsulation efficiency of ca. 95% after a two-step emulsification process. The WPI/XGH complex had a synergistic effect on the emulsifying properties compared with the biopolymers alone, and they could act as a thick and efficient barrier against the release of flumethrin.

3.3 Stabilization by solid particles

Apart from biopolymers and their complexes, solid particles (such as paraffin, triacylglycerols, polymers, or clay) can generate a network and be adsorbed at the interface between different phases, providing a physical barrier to inhibit fusion(Low, Siva, Ho, Chan, & Tey, 2020; Szumała & Luty, 2016). Such kind of stabilization mechanism is termed as Pickering stabilization, and the as-prepared emulsions are named Pickering emulsions(Dupont, Maingret, Schmitt, & Héroguez, 2021). Since the irreversibly adsorbed solid particles can function at the oil-water interface by controlling the wettability and contact angle of the solid colloidal particles, the Pickering emulsion possesses higher stability (resistance to deformation) than conventional emulsions for the long-term storage (Low, et al., 2020; H. Yu, et al., 2021). Liu et al.(Yuan, et al., 2021) explored the preparation of stable O/W/O Pickering emulsions for the formulation of Pickering emulsion hydrogel microbeads entrapping copper extractants. Given the fact that the cross-contamination ratio (the degree of migration between inner and outer oil phases) of O/W/O Pickering emulsions could be reduced to <3% under pre-set conditions, it suggested that further improvement of the stability and preventing the cross-contamination between internal and external phases is possible. In the food science field, tiny fat crystals can be considered as solid particles to stabilize the oil-water interface, forming Pickering emulsions(G. Li, et al., 2021; Ashok R. Patel, 2017; A. R. Patel & Dewettinck, 2016; V. Patel, Andrade, & Rousseau, 2021). Patel(Ashok R. Patel, 2017) chose the mixture of melted palm oil and different concentrations of sunflower oil (POSO) as an external phase to prepare O/W/O emulsions. The formulated O/W emulsions were homogenized with melted POSO, after which the temperature decreased to 5 °C using an ice bath. With the temperature decreasing, fat crystals were generated and accumulated in the oil-water interface, following which the formed bulk crystallization stabilized the O/W/O emulsion droplets through physical entrapping in the fat crystal network. It is worth noting that the properties of fat crystals (i.e. mean size, shape, morphology, and crystal compositions) play a crucial role in the stability of the emulsions

(Cheng, Kan, Cao, Dudu, & Yan, 2021; Dickinson, 2010b; Ghosh & Rousseau, 2011; Rousseau, 2013; Yang, et al., 2020).

3.4 Stabilization by viscosity

Controlling the mobility of the different phases in an O/W/O double emulsion tends to prevent flocculation, coalescence, and creaming, as well as reduce the delivery rate of the inner oil from the internal phase to the external phase through the interlayer. To address the issues, some approaches have been proposed: (1) adding polymers into the intermediate aqueous phase; (2) increasing the viscosity or solidification of the inner and/or outer oil phase(Y.H. Cho, 2003).

Viscosity is an important parameter for emulsion stability. Reportedly, the increased viscosity of the water phase could markedly improve the resistance of dispersed water droplets towards approaching each other(K. Zhang, et al., 2020). Polysaccharides can be considered as stabilizer/thickening agents for increasing the viscosity of the aqueous phase in the O/W emulsion system, thus enhancing the emulsion stability by restricting the movement of oil droplets(Ozturk & McClements, 2016). This has been demonstrated by adding the xanthan gum into O/W emulsions(Boonlao, Shrestha, Sadiq, & Anal, 2020; Matsuyama, et al., 2021; Piroozian, et al., 2021; Xiao, et al., 2021). It was also reported a high concentration of starch can improve the viscosity of the aqueous phase and then strengthen the emulsion stability(Zang, Wang, Yu, & Cheng, 2019). A successful case is that native corn starch (7 wt%) was utilized to formulate the O/W/O emulsions forming a strong network structure that suppressed the movement of the droplets and reduced the collision frequency. Besides, a high concentration of the starch could also strengthen the viscosity of the double emulsion system, which in turn reduced the creaming phenomenon and kept stable for 2-week storage(Yang, et al., 2021). Similar observations were also reported elsewhere(Cai, et al., 2021; Z. Li, et al., 2020; Xu, et al., 2020).

Similarly, the viscosity and density of the oil phase in a W/O emulsion also affects the droplet breakup and mobility, which further influences the stability of the emulsion(Kumar, Roy, Devra, Dhiman, & Prabhakar, 2021). Ushikubo and Cunha(Ushikubo & Cunha, 2014) found that W/O emulsions prepared with hexadecane possessed lower stabilization than that formed with soybean oil, the behind reason of which could be due to the fact that the viscosity of hexadecane was lower than soybean oil, resulting in a higher sedimentation rate and a faster phase separation. Furthermore, the higher interfacial tension at the water-hexadecane interface and the larger difference in density between water and hexadecane could bring about a faster phase separation of the as-prepared emulsions(Ushikubo & Cunha, 2014).

Solidification has been recognized as another approach for improving the stability of an O/W/O emulsion. It restricts the migration of the internal phase to the external oil phase, which generally occurs in the process of fat spread and drug delivery(Gaonkar, 1994). In order to realize long-term storage of the as-formed O/W/O emulsion, a certain amount of solid fat with special morphology is required allowing the external phase to generate a crystal network around the O/W emulsion droplets(Firouz Jahaniaval, 2003; Pradhan & Rousseau, 2012; Thanh Diep, et al., 2018). As an example, a certain concentration of candelilla wax was dissolved into soybean oil serving as the outer oil phase for the formation of an O/W/O double emulsion. By adjusting the storage temperature, the outer waxy oil phase was crystallized and solidified. The result is that the as-fabricated O/W/O emulsion with this method was more stable at 4 ºC than at the high temperatures of 20 ºC and 40 ºC(S.-C. Yu, et al., 2003). Diep et al. formulated O/W/O emulsions using the mixture of palm oil and sunflower oil (serving as oil phase), after which the emulsion was stored in thermal cabinets at different temperatures(Thanh Diep, et al., 2018). The microscopic results indicated that the structure of the O/W/O emulsion was rapidly destroyed after storage at 15°C for 24 hours. When it was stored at 10°C, the sample could be well remained stable for one week. Nonetheless, some of the O/W droplets were merged and larger droplets were formed as confirmed from the optical microscopy. Impressively, any structure changed was not identified when the double emulsion was stored at 5°C after 4 weeks.

4 Approaches for stability evaluation of the O/W/O emulsions

Studying the structure of O/W/O emulsion and their inner and outer droplets is crucial for understanding and predicting the stability of the as-prepared emulsion. The present methods for assessing the stability of O/W/O double emulsions are mainly based on advanced microscopy, light scattering, rheology, and labeling assays(Aguiar, et al., 2020; Leister & Karbstein, 2020).

Microscopy can be used to unveil the formation process of O/W/O emulsions, the morphology of droplets, the mean droplet size, and its variation, as well as the degree of filling. All these factors are closely related to the emulsion stability(Qin, et al., 2021; Ren, et al., 2021; Yuan, et al., 2021). By referring to a self-assembled visualization system, including microscopy, a high-speed camera, a computer, and a light source, Lu et al. recorded the transient formation processes of single-core and multi-core O/W/O double emulsions and determined their filling degree by a co-flowing microfluidic device(Lu, Wu, & Liu, 2017). Using optical microscopy, Hwang et al. observed the effect of the content of surfactants and polymers added into the water phase on the stability of spherical silica particles encapsulating the retinol, which were formulated by the O/W/O emulsion and a sol-gel method(Hwang, et al., 2005). Field emission scanning electron microscopy (FE-SEM) was utilized to explore the aggregation of spherical silica particles in O/W/O emulsions and the influence of the involved polymers on the morphology. Pradhan et al. adopted a one-step protocol to prepare O/W/O emulsions, the morphology of which was collected by confocal laser scanning microscopy (CLSM) and differential interference contrast microscopy. Moreover, the two methods were also utilized to confirm the formation of O/W/O emulsions and visualize droplet size evolution over time, respectively(Pradhan & Rousseau, 2012). It depicted that the migration of inner oil droplets to the outer oil phase through hole propagation. However, because of the limits of the microscopy, only qualitative analysis can be carried out.

Light scattering is an important tool for measuring the droplet size of an emulsion as well as its change over time. It allows us to calculate the degree of flocculation (FD) and coalescence (CD) and evaluate the emulsion stability(J. Li, et al., 2020; Santos, Calero, Trujillo-Cayado, Alfaro, & Muñoz, 2018; Su, et al., 2020; Wang, Zhang, Wang, Xu, & Jiang, 2020). Su et al. formulated lutein Pickering emulsions stabilized by β-lactoglobulin-gum arabic nanoparticles. The long-term storage stability of the emulsions was examined by recording the changes in emulsion droplets over 12 weeks using light scattering. Moreover, the FD and CD were also calculated(Su, et al., 2020). However, this measure is generally carried out for primary O_1/W emulsions before the second emulsification step.

As is well known, the rheological behavior could also significantly influence the stability of emulsions, the reason of which should be mainly attributed to the internal structure and its composition(Liu, et al., 2018). Viscosity is usually determined by rheometers and is directly related to the proportion of dispersed phase and the droplet size distribution of the emulsions(Malkin, Masalova, Slatter, & Wilson, 2004). In Krieger–Dougherty model, the viscosity of a liquid emulsion can be described as a function of the fraction of dispersing phase(Rahim, Milad, Yusoff, Airey, & Thom, 2021). The theory is also suitable for O/W/O double emulsions(Lutz, Aserin, Wicker, & Garti, 2009). Yang et al. added different amount of native starch to the aqueous phase of O/W/O double emulsions toward understanding the effect of formulation differences on the stability and structure of the emulsions(Yang, et al., 2021). As a result, O/W/O double emulsions with 7% native corn starch in the aqueous phase exhibited a higher viscosity compared to other emulsions with counterpart low concentration. It suggests that the high concentration of starch increases the coordination relationships between the molecular chains and changes the intermolecular interactions, subsequently inhibiting the aggregation between the emulsion droplets. Of note, a high concentration of starch can thicken the aqueous phase, resulting in a tight three-dimensional mesh architecture, which improves the rheological properties and emulsion stability(Krstonošić, Dokić, Nikolić, & Milanović, 2015).

The encapsulation efficiency and targeted release of active substances in O/W/O double emulsions can be applied to evaluate whether phase transformation is occurred, which is also an important means for evaluation of the emulsion stability, where detection methods include infrared spectrophotometry, differential scanning calorimetry (DSC), confocal laser scanning microscopy(CLSM) and nuclear magnetic resonance (NMR) characterizations(C. Laugel 2000; Leister & Karbstein, 2020; McClements, 2015; Svanberg, Wassen, Gustinelli, & Ohgren, 2019). Inspired from the Krieger–Dougherty equation, a method was developed to calculate the encapsulation efficiency of O/W/O double emulsions using the viscosity parameters, whose calculation results were consistent with PFG-NMR measurement(Schmidt, Bernewitz, Guthausen, & Schuchmann, 2015). Due to the different diffusion behavior, PFG-NMR approach can well differentiate the phases of O/W/O double emulsions. For a spectral differentiation between water and oil, it can be detected with high field measurements. Also, it was noticed that high performance liquid chromatography (HPLC) was suitable for investigating the *in vitro* retinol release and EE of retinol in an O/W/O emulsion(Hwang, et al., 2005).

In addition, the filling degree of the aqueous phase could directly affect the overall density of O/W/O double emulsions, leading to the change in the settling rate of the primary O_1/W droplets. The encapsulation efficiency can be determined by this method as well(K. Pays, 2001). Both the settling rate and the post-settling phase fraction can be used to evaluate the stability of O/W/O double emulsions. Once the inner oil phase was transferred through the aqueous phase to the outer oil, the total volume of O_1/W primary emulsion droplets will be less, conversely, the volume of the external oil phase will increase. The increase in the volume fraction of the separated oil phase after settling is therefore the amount of oil that released from the inner oil phase. Different from the natural settling that generally takes long time, centrifuge treatment is generally adopted to speed up the process. Balcaen et al. proposed a straightforward method to investigate the capsulated water fraction of W/O/W double emulsions using LUMiSizer dispersion analyzer(Balcaen, Vermeir, Declerck, & Van Der Meeren, 2015). This method could also be effective for the determination of the migration amount of the internal oil phase in O/W/O double emulsion.

5 Potential applications in the food industry

O/W/O double emulsions have great potential in the food industry for the following purposes: reduction of trans- and saturated fat content, prevention of deterioration of oxygen-sensitive ingredients, flavour encapsulation, nutrient delivery, and controlled and targeted release of functional components. To date, tremendous attentions have been paid for real application of double emulsions for food industry, while there are few patents and literature yet.

O/W/O double emulsions were originally applied in the pharmaceutical field. It was reported that the pentazocine in O/W/O emulsions allows more effective drug release than the counterpart simple O/W emulsions(Pandit, 1989). For food fabrication, the double O/W/O emulsion system was mainly suggested for the manufacture of spreads(Firouz Jahaniaval, 2003; Gaonkar, 1994). Table 1 lists the application cases of the O/W/O emulsion in the food field. Gaonkar constructed O/W/O double emulsions as butter flavor in the inner phase for low-fat spread, which provided a possibility for encapsulating edible essential oil in O/W/O emulsion structure(Gaonkar, 1994). Jahaniaval et al. proposed a novel approach to formulate low-fat products with an O/W/O emulsion structure, which was stabilized with 4% palm and cotton stearin by blending with primary O/W emulsions followed by a supercooling treatment(Firouz Jahaniaval, 2003). To improve the stability and texture of spreads with an O/W/O emulsion structure, other studies were also carried out and reported elsewhere(Dwyer, O'Beirne, Ni Eidhin, & O'Kennedy, 2012; O' Dwyer, O' Beirne, Ní Eidhin, Hennessy, & O' Kennedy, 2013; O’Dwyer, O’Beirne, Ní Eidhin, Hannon, & O’Kennedy, 2013; Ashok R. Patel, 2017). A study on sensory testing of low-fat foods showed that replacing fat with internal water droplets did not markedly alter the perceived intensity of fat-related properties, which has positive implications for developing food products with O/W/O emulsion system(Oppermann, Piqueras-Fiszman, de Graaf, Scholten, & Stieger, 2016). Therefore, O/W/O double emulsions can be recognized as fat replacers for improving the water content in foods without changing the sensorial properties. Also, a unique O/W/O emulsion structure provides a new pathway for production of bakery fat with required texture and healthy ingredients. Apart from low-fat foods, another important role in the development of the

O/W/O emulsion is its efficient delivery of nutrient and flavour components as the emulsion favours the controlled release of active ingredients. By incorporating polyphenolic extract, Katsouli et al formulated an O/W/O double emulsion with enhanced stability(Katsouli, Giannou, & Tzia, 2020). In this system, coenzyme Q10 or conjugated linoleic acid was loaded and their kinetic and chemical stability was improved. Besides, a concentrated O/W/O emulsion model was successfully elaborated and optimized by encapsulating two model fragrances composed of 10 and 13 representative molecules, after which a third fragrance was used to test the stability and feasibility. The results exhibited the encapsulation efficiency of the selected O/W/O system was close to 99%(Stasse, et al., 2020).

6 Conclusion

Due to human's high desire for a healthy diet, reducing fat intake and replacing trans- and saturated fatty acids has become a popular trend, among which structuring edible oils has been being a particularly interesting topic. One of the frontier topics in structured fats and oils is the double emulsion, which possesses a special structure typically achieved by multiple-interface stabilization; as reported, it can capture large amounts of liquid oils within a single internal space. Because of the structure features, the O/W/O emulsion is also suitable for efficient nutrient delivery and flavour delivery. In this work, the recent development of O/W/O double emulsions (over the past 20 years) are reviewed, mainly being focused on the formulation of the O/W/O emulsion, stabilization mechanisms, and its potential applications. Although some big progresses have been achieved so far, there are still some obstacles that need to be addressed, such as the primary W/O emulsion breakage due to strong forces during the second emulsification step and product instability during storage, as well as texture control difficulty under different processing conditions. Given the fact that the O/W/O double emulsion could be a promising vehicle for nutrient delivery in food, further study on how they function in the human body when delivering nutrients during digestion is needed. Noteworthy, research on the O/W/O double emulsion is currently in the experimental stage in laboratory, achieving large-scale industry application is still on the way, but should be accelerated.

Table 3.1 Overview of the recently reported O/W/O double emulsion and their applications in the food field

Inner oil phase	Aqueous phase	Outer oil phase	Applications	Markers
Oil-soluble flavors	Aqueous soluble/gellable polysaccharides with a hydrophilic emulsifier	Edible triglyceride oils derived from seed oils containing lipophilic emulsifier	Low-fat spreads comprising flavors	Stable multiple emulsions comprising interfacial gelatinous layer were patented(Gaonkar, 1994).
Liquid canola oil	Water with sodium caseinate	Liquid canola oil and palm-cotton stearin containing lecithin	Low solid fats	A novel method to prepare O/W/O emulsions was created(Firouz Jahaniaval, 2003).
Camelina/fish (85/15) oil blend	Water containing sodium caseinate and green tea extract (GTE)	Palm oil/rapeseed oil blend containing polyglycerol polyricinoleate (PGPR) and α-Tocopherol	Omega-3 rich spreads	GTE could enhance the chemical stability of O/W/O double emulsions rich in omega-3 during storage(Dwyer, et al., 2012).
The mixture of camelina oil and fish oil	Water containing sodium caseinate and NaCl	The mixture of palm oil and sunflower oil containing PGPR	Table spreads	After being encapsulated into O/W/O emulsions, omega-3 fatty acids possessed better oxidative stability(O' Dwyer, et al., 2013).
Sunflower oil	Water containing gelation and xanthan gum	Melted Palm oil without or with sunflower oil	Table spreads and cooking fats	The inner oil droplets were stabilized by the gelation of polysaccharides while the water ones were stabilized by fat crystals and the fat network they formed(Ashok R. Patel, 2017).
Liquid paraffin including retinol	Water containing 1,3-butanediol with HCO-60	Oil gel comprising organophilic clay mineral and emalex 600 di-IS or 600 di-O	Nutrient delivery	The stability of retinol encapsulated in O/W/O emulsions was higher than that in W/O or O/W emulsions(Katsunori Yoshida, 1999).

Inner oil phase	Aqueous phase	Outer oil phase	Applications	Markers
Spherical silica particles containing retinol	Water containing Tween 20, NH_4OH, and polymer	n-decyl alcohol including hydroxypropyl cellulose and Span 80	Nutrient delivery	Adding poly(ethylene glycol)-block poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic P123) into the water phase could cause a higher release degree and encapsulation efficiency of retinol than polyvinylpyrrolidone or polyethylene glycol(Hwang, et al., 2005).
Extra virgin olive oil (EVOO) or olive pomace oil (OPO) containing conjugated linoleic acid (CLA) or CoQ_{10}	Water containing polyphenols from the olive kernel and Tween 40	EVOO or OPO containing Span 20 and Tween 40	Nutrient delivery	O/W/O double nanoemulsion system could protect CLA and CoQ_{10} presenting high retention during storage(Katsouli, et al., 2020).
Sunflower oil containing lycopene	2.0% sodium alginate solutions with soy protein isolate (SPI)	Sunflower oil containing Span 80	Nutrient delivery	During intestinal digestion, the emulsion micro-gel particles formulated by the internal gelation method is much more stable than those by the external method, therefore slowing down the release rate of encapsulated lycopene(Lin, Kelly, & Miao, 2021)
Corn oil	Water containing whey protein isolate	Corn oil containing PGPR	Delivery system	This O/W/O double emulsion could protect the internal oil from oxidation and control the digestibility of lipid droplets within the gastrointestinal tract(Sung, Xiao, Decker, & McClements, 2015).
Flavor mixture	water with maltodextrin and gum arabic	Molten hydrogenated palm kernel oil containing 5% emulsifier	Flavor encapsulation	The structure of emulsifiers highly influenced the stability of O/W/O emulsions and the mixture of Span 80 and PGPR showed better stability than single PGPR(Y.H. Cho, 2003).
Model fragrances	Water containing NaCl and hydrophilic surfactants (Tween 60, Tween 20, or Tergitol 15-S-12)	Isopropyl myristate containing PGPR or Span 80	Fragrance encapsulation	A unique formulation of O/W/O structure was suitable for encapsulating a large amount of fragrance and inhibiting fragrance deterioration(Stasse, et al., 2020).

Inner oil phase	Aqueous phase	Outer oil phase	Applications	Markers
Satureja hortensis essential oil (SEO)	Water containing alginate solution and Tween 80	Sunflower oil containing Span 80	Essential oil encapsulation	SEO-loaded microparticles could successfully be formulated by o/w/o emulsions/ionic gelation technology(Hosseini, et al., 2013).
Fish oil (FO)	Water containing succinic acid deaminated wheat gluten (SDWG)	Mineral oil with 1% Span 80	Supplementation and functional foods	A delivery system encapsulating FO was formulated using traditional two-step emulsification and subsequent heat-polymerization(Liao, et al., 2012).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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