Development of stable curcumin nanoemulsions: effects of emulsifier type and surfactant-to-oil ratios

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Abstract Curcumin, a natural polyphenolic compound, offers a wide range of pharmacological benefits such as antioxidant, anti-inflammatory and anti-cancer. The oil-in-water nanoemulsions containing curcumin were obtained by high pressure homogenization and effects of various emulsifiers (Tween-80, lecithin, whey protein isolate and acacia) and different surfactant-to-oil ratios (SOR) on physicochemical characteristics, physical stability and storage stability of curcumin loaded nanoemulsions were evaluated in this study. The result showed that smaller particle size, better physical and storage stabilities and higher curcumin content were found in curcumin loaded nanoemulsions stabilized with Tween-80 and lecithin. Compared with nanoemulsions prepared with lecithin, nanoemulsions fabricated with Tween-80 exhibited better uniformity and distribution as demonstrated by microscopic observations. It was found that SOR was positively correlated with particle size but negatively correlated with curcumin content in the emulsion droplets. Neither the emulsifier nor SOR values were found to have significant effects on zeta-potentials of the droplets. This result implied that curcumin loaded nanoemulsions prepared with Tween-80 and higher SOR values helped curcumin to achieve better physical stability and storage stability.

Keywords Curcumin · Nanoemulsion · Emulsifier · Surfactant-to-oil ratios · High pressure homogenization

Introduction

Curcumin is a kind of edible yellow pigment extracted from the rhizome of zingiberaceae plants such as turmeric. Its dyeing capacity is greater than other natural pigments and synthetic lemon-yellow pigment. In addition, curcumin can be used for coloring medicines, cosmetics and foods (Hashem et al. 2010). As a kind of Chinese traditional medicine, turmeric contains many curcumins. Therefore, despite for coloring, curcumin can also be used in food additives because of its nutritional and pharmacological functions. Studies have demonstrated that curcumins are efficient scavengers for their superoxide scavenging activity (Kunchandy and Rao 1990). In the past 50 years, curcumin has been proved to be effective in both preventing and treating cancer, antirheumatic, anti-inflammatory and treatment of diabetes (Aggarwal et al. 2003; Deodhar et al. 2013; Kaur and Das 2011; Weisberg et al. 2008). What's more, curcumin has also been reported to have antiradiation function (Xie et al. 2014).

Emulsions are multiphase dispersion where a liquid dispersed in another immiscible liquid. In recent years, many emulsifiers are used to prepare nanoemulsions, such as small molecule surfactants, phospholipids and proteins (Chang and McClements 2016). However, various emulsifiers may lead to the different types of emulsion systems, while researches on such comparison are limited. The phase in form of the liquid droplets is called dispersed phase, while the other phase is called the dispersion medium (Arshady 1992). The basic types of emulsions fall into two categories, including oil-in-water (O/W) and water-in-

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oil (W/O). There are many factors that influence the emulsion types, such as the nature of the oil and water phases, the ratio of water to oil, temperature, the structure and nature of emulsifiers, agitation and shearing impacts (McClements 2012). The study on emulsions starts late. However, it popped up everywhere and played an irreplaceable role in practical products, such as paint, cream, metal cutting fluid and oil-based drilling fluids (Frasch-Melnik et al. 2010; Bjerregaard et al. 2001). An emulsion helped the encapsulated active ingredients to achieve a controlled and sustained release, which alleviated the side effects caused by the direct contact between the tissue and the bulk of the drug (Chemtob and Asua 2013).

Clinical trials have shown that curcumin can be used safely in the human body. However, curcumin's poor bioavailability resulted in many challenges for its applications (Anand et al. 2007; Young et al. 2014). Studies to improve the bioavailability and stability of curcumin have been conducted. For example, the emulsion–diffusion–evaporation method was applied to prepare the curcumin loaded nanoparticles and curcumin showed better bioavailability in nanoparticles than in powders (Shaikh et al. 2009). Moreover, many studies solved the low absorption problem of curcumin by preparing liposomes, phospholipid complexes and curcumin-metal chelates (Liu et al. 2006).

An emulsion system can increase the solubility and bioavailability of curcumin and other bioactive compounds, such as beta carotene and lutein (Liu et al. 2016; Xu et al. 2016). Many factors such as temperature and light affected the stability of the emulsion system. Previous researches showed that polysaccharides and phospholipids can be used to encapsulate curcumin within complexes (Tønnesen et al. 2002). Due to the low water solubility, curcumin was embedded in liposomes to enhance the bioavailability and solubility (Ahmed et al. 2012). Based on exploring influence of oil phase on nanoemulsions in previous studies, the present work further discussed the impacts of different emulsifiers on the curcumin loaded nanoemulsion system. In addition, the effects of oil types and surfactant-to-oil ratios (SOR) on the stability of curcumin-loaded nanoemulsions were compared in this work, which aims to provide theoretical basis for improving the physicochemical characteristics of curcumin loaded nanoemulsions.

Materials and methods

Materials and chemicals

Curcumin (95% purity, Tianxu Biological Technology Co., Ltd, Hebei, China), medium-chain triglyceride oil (MCT,

Guangdong Wincom Flavors & Fragrances Co., Ltd, Guangdong, China), canola oil (Canola Harvest, Alberta, Canada), linseed oil (ZhangbeiBaosheng Oil Co., Ltd, Hebei, China), sunflower oil (JinyeASTCo., Ltd, China), corn oil (Sanxing Corn Industry Science and Technology Co., Ltd, China), lecithin (food grade, De City Food Products Factory, He'nan, China), acacia (TIC GUMS, Guangdong, China), Tween-80 (Xilong Chemical Industry Co., Ltd, China), whey protein isolate (WPI, Davisco Foods International, Inc., Eden Prairie, Minnesota, USA), alcohol (95%) and ethanol (99.99%) were acquired from Beijing Chemical Works (Beijing, China).

The standard curve of curcumin content

The solubility of curcumin was determined following the previous procedures. Briefly, 6 mg of curcumins were dissolved in alcohol to make up 100 mL of solutions at room temperature (Ma et al. 2017). Aliquots of 250, 500, 1000, 2000, 3000, and 4000 μ L from the solution separately were extracted and diluted to 15 mL with alcohol. The absorbance of such dilutions at a wavelength of 425 nm was determined using a UV spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Each set of samples was determined three times in parallel. Finally, the standard curve of UV absorbance against curcumin concentration was plotted.

Solubility of curcumin in different oils

The solubility of the curcumin is tested in five different oils (MCT, canola oil, sunflower oil, linseed oil, and corn oil) at room temperature. Curcumin (1.5 g) was dissolved into 100 mL five different oils and then three methods were used to make curcumin dissolve completely, including, thermal treatment (stirring in boiling water bath for 3 min), sonication (390 W, 30 min, interval 1 s, JY32-11N Ultrasonic homogenizer, Ningbo Scientz Biotechnology Co. Ltd, China) and microwave treatment (780 W, 30 s, NJL07-3 Microwave experimental furnace, Nanjing Jiequan Microwave Equipment Co., Ltd, China) (Mao et al. 2010). All the 15 mixtures were centrifuged at 3000 rpm for 2 min, and the supernatants were collected. All the collected solutions were kept at room temperature in dark for 24 h before analysis (Ma et al. 2017).

Preparation of emulsion system

According to the results of curcumin solubility, three kinds of oils (canola oil, linseed oil and MCT) in which curcumin showed a relative high solubility were selected as oil phase in nanoemulsions. Compared to thermal and microwave methods, sonication was much easier to dissolve curcumin

into oils, which was chosen to dissolve curcumin. Then, four emulsifiers of lecithin, acacia, Tween-80 and WPI were used for preparation nanoemulsions. Briefly, curcumin (3 g) were separately dissolved into 250 mL of three oils mentioned above, and the mixtures were sonicated (JY32-11N Ultrasonic homogenizer, Ningbo Scientz Biotechnology Co. Ltd, China) under 390 W for 30 min with intervals of 1 s. Then the samples were centrifuged at 3000 rpm for 2 min. The supernatants were collected and kept at room temperature for 24 h. Tween-80 (2 wt%), lecithin (2 wt%), acacia powders (2 wt%) and WPI (2 wt%) were dissolved in deionized water separately and stirred for 4 h until adequate hydration. The dispersions were then diluted to 1000 mL with deionized water in volumetric flasks. Aliquots of the three oil solutions of curcumin was extracted and mixed with the aqueous solutions of emulsifiers (5, 10, 15, 20, 25, and 30 wt%) correspondingly. The final mixtures were blended using an Ultra-Turrax T25 high-speed blender (IKA, Staufen, Germany) at 10,000 rpm for 6 min to form coarse emulsions. Those coarse emulsions were then homogenized at 60 MPa using a high-pressure homogenizer (Type NS 1001L2K, GEA Niro Soavi S.p.A, Parma, Italy) for three times at ambient temperature to produce nanoemulsions (Mao et al. 2010). Sodium azide (0.02 wt%) was added as an antimicrobial agent.

Determination of the content of curcumin

Aliquots of the curcumin oil solutions and the nanoemulsions were extracted and diluted for 1000 times with alcohol (95%). The absorbance at a wavelength of 425 nm was determined using a UV spectrophotometer (Safie et al. 2015). All determinations were performed in triplicate and the curcumin content of each sample was calculated according to the standard curve (standard curve line: $Y = 0.0146X + 0.0222$, $R^2 = 0.9993$). The alcohol solution (95%) was used as control.

Determination of zeta-potential and particle size

Zeta-potential and particle size of the curcumin encapsulated nanoemulsions were determined with Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) (Abbas et al. 2014). Samples were equilibrated for 60 s after placing in the instrument, and the reported data was collected as the mean of over 30 continuous readings. Emulsions diluted with deionized water for 500 times are used for particle size (represented by mean particle diameter) determination and those diluted for 1000 times with 95% alcohol were used for zeta-potential determination to avoid the effect of multiple light scattering (Sari et al. 2013). All determinations were performed in triplicate.

Determination of physical stability

The stability of the O/W emulsions was analyzed with LUMiSizer (L.U.M. GmbH, Berlin, Germany). Emulsions (1.8 mL) were loaded into a centrifuge tube. The samples were centrifuged at 4000 rpm for 7650 s with an interval of 30 s. The measurements were performed at room temperature and the emulsion stability was shown as a space- and time-related transmission profiles over the sample length (Tan et al. 2016a, b).

Cryo-scanning electron microscopy (Cryo-SEM)

Cryo-SEM (Philips CM12, Philips, Eindhoven, Netherlands) was used to qualitatively characterize the droplet size of the prepared emulsions. Emulsions were diluted to 0.5 mg/g of dispersed phase with 10 mmol/L citrate buffers (pH 3.6). One drop of the diluted emulsions was placed on a copper grid that was then quickly transferred to a liquid nitrogen bath for solidification. The copper grid was then transferred to the SEM cold stage using a cryo-holder. The stage was kept under $-170\text{ }^{\circ}\text{C}$ with liquid nitrogen (Sun et al. 2017).

Determination of storage stability

The curcumin contained nanoemulsions were stored for 60 days and the curcumin content and particle size of the droplets were determined by the UV spectrophotometer and Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) to reveal characteristics changes during the storage period (Ma et al. 2017).

Statistical analysis

All data were collected from triplicates and were reported as the average with standard error. Data were analyzed by one-way analysis (Duncan test) of variance using the SPSS 16.0 package (SPSS Inc., Chicago, USA).

Results and discussions

Influence of emulsifiers

The interfacial activity of emulsifier plays an important role in the characteristics and stability of nanoemulsions (Schubert and Engel 2004). Encapsulation of poor water-soluble curcumin expanded the utilization of curcumin in food industry (Sheikhzadeh et al. 2016). In this study, we discussed the influence of emulsifiers from two aspects: the species and concentrations of the emulsifiers.

Influence of emulsifiers on characteristics of curcumin loaded nanoemulsions

Curcumins are fat-soluble pigments and have low solubility in water (Tønnesen et al. 2002). In this experiment, curcumins were dissolved in different types of oils and treated with different methods to increase their solubility in the oil phase.

Figure 1 shows the curcumin solubility in various oil phases treated by different methods. On one hand, it can be concluded that compared with microwave treatment, ultrasonic treatment had statistically insignificant effects to heat treatment on the curcumin solubility in ice-bath. Room-temperature preparation is usually more preferable in food industry. Therefore, ultrasonic treatment was selected as the method for further research. It also showed in Fig. 1 that species of the oils affects the curcumin solubility in nanoemulsions. The curcumin solubility in the nanoemulsions prepared with MCT as oil phase and Tween-80 as emulsifier (30 wt%) could reach to 12 mg/L, but which in the nanoemulsions prepared with canola oil was 4 mg/L. It is obvious that MCT is the best solvent for curcumin while canola oil is the worst one regardless of the dissolution treatments. Curcumin solubility showed statistical insignificant differences between sunflower oil, linseed oil and corn oil. This can be explained by different interactions and excluded volume effects (Huyskens and Haulait-Pirson 1985). Longer-chain triglycerides per unit mass have less polar groups, which may decrease the occurrence of dipole–dipole interactions between dipole

Fig. 1 Effect of different dissolution methods and oil types on curcumin content. Red means high value (color figure online)

polar group and curcumin molecules, and it is beneficial to increase solubility. Excluded volume effect is another factor for the solubility of curcumin. There are always some curcumin molecules outside the lipid system, so the concentrate in this zone is zero. And concentrate is related to osmosis effect and is thermodynamically stable, which influences curcumin's solubility in oil phase.

Figure 2 shows the characteristics of curcumin nanoemulsions. Four kinds of emulsifiers were used to prepare curcumin loaded nanoemulsion, including Tween-80 (HLB = 15), lecithin (HLB = 12), WPI (HLB = 9) and acacia (HLB = 8), representing small molecule surfactants, phospholipids, proteins and polysaccharides, respectively. Curcumin content increases with the concentration of oils in the nanoemulsions, regardless of the species of the emulsifiers (Fig. 2a–c). In the nanoemulsions prepared with MCT as the oil phase, it was seen that the systems using Tween-80 and lecithin as surfactants obviously increased the curcumin solubility than those using WPI and acacia. Meanwhile, the average particle size of the nanoemulsions using WPI as emulsifiers is relatively larger than the others. Moreover, it is worth noting that the sizes of droplets with Tween-80 as emulsifiers were commonly smaller than 300 nm, and the diameter of the smallest droplets made up from linseed oil (10%) was only 193.8 nm (Fig. 2d–f). The result shows much smaller than other likeness curcumin nanoemulsion such as 633 nm (Pathak et al. 2015). Compared with other emulsifiers, lecithin and Tween-80 provided relatively good properties to the nanoemulsions, because generally speaking higher curcumin content and less particle size are better properties to curcumin loaded nanoemulsions. These results can probably be explained by different hydrophilicity of the emulsifiers. High hydrophilicity and high hydrophobicity emulsifiers can make the O/W system more stable, resulting in smaller droplet size (Tan and Nakajima 2005). The hydrophilicity of an emulsifier is usually represented by its hydrophilic-lipophilic balance (HLB) value. In this study, the HLB values of Tween-80 and lecithin were 15.0 and 12.0, respectively, which means Tween-80 was more hydrophilic than lecithin. Therefore, the O/W systems prepared with Tween-80 as emulsifiers exhibited smaller particle size than those prepared with lecithin.

Figure 3 shows the microstructure of nanoemulsion droplets containing curcumin. Compared with Fig. 3a2, it can be seen in Fig. 3a1 that the droplets displayed higher monodispersibility, indicating that curcumin nanoemulsions prepared with Tween-80 performed better than those with lecithin. When magnified 5000 times as shown in Fig. 3a, curcumin nanoemulsions prepared with Tween-80 were found to have droplets similar in size, while the droplets of those prepared with lecithin aggregated. It was more obvious when the images were magnified 50,000

Fig. 2 Effect of emulsifier type treatments at different oil concentrations: **a** MCT as oil phase, content variation; **b** canola oil as oil phase, content variation; **c** linseed oil as oil phase, content variation; **d** MCT as oil phase, size variation; **e** canola oil as oil phase, size variation;

f linseed oil as oil phase, size variation; **g** MCT as oil phase, zeta-potential variation; **h** canola oil as oil phase, zeta-potential variation; **i** linseed oil as oil phase, zeta-potential variation. Red means high value (color figure online)

times, as shown in Fig. 3b. The droplets of nanoemulsions prepared with Tween-80 were much smaller than those emulsified by lecithin. This result is in accordance with previous conclusions (Lin et al. 2009). Such difference is more significant at higher concentration of the oil phase as shown in Fig. 3c. In the case of Tween-80 as the emulsifier, although substantial aggregations were found, the

nanoemulsions were still well dispersed compared with those prepared with lecithin where higher extents of droplet aggregations were observed. Emulsifiers reduced the interfacial tensions between different phases, allowing the droplets to be stable (Züge et al. 2013). Smaller droplets will create more interfacial areas. Thus, when the droplets

Fig. 3 Influence of different oil concentrates and types on droplets microstructure. MCT as oil phase and microstructure was observed by electron microscope: **a** tween-80, ratio of oil 5 wt%; **b** lecithin, ratio of oil 5 wt%, scale bar-1 μm ; **c** tween-80, ratio of oil 25 wt%; **d** lecithin, ratio of oil 25 wt%; (1) and (2) is same nanoemulsion under different scale, **e** the physical stability of curcumin emulsions formed with tween-80 and lecithin as emulsifier, expressed as the integrated transmission time plots obtained by the LUMiSizer

are smaller and more dispersible, the emulsion will be more stable (McClements 2007; Schramm 1992).

Influence of emulsifiers on physical stability of curcumin nanoemulsion

Physical properties of an emulsion such as stability, rheology and optical characteristics or mass-transfer kinetics are influenced by its characteristic microstructure. Therefore, it is not surprising that there is significant ($p < 0.05$) difference between nanoemulsions prepared with different emulsifiers.

Figure 3e shows the integrated transmission-time plots for various curcumin contained nanoemulsions. It can be concluded that those curcumin nanoemulsions prepared with Tween-80 performed better physical stability than the others regardless of the surfactant-to-oil ratios (SOR) values. Under the condition of high-speed centrifugation, the lighter oil phase moved to the top and the heavier part moved to the bottom, improving the transmission of nanoemulsions (Yuan et al. 2013). According to the Stokes' law, larger values of viscosity lead to slower floating rate of emulsion droplets, which also means better physical stability (Walstra and Vliet 1996). Therefore, it can be clearly concluded from the results that curcumin nanoemulsions prepared with Tween-80 as emulsifiers possessed better physical stability. This conclusion shared by different types of oils and detailed discussion in previous study (Ma et al. 2017).

Influence of emulsifiers on storage stability of curcumin nanoemulsion

Storage stability of nanoemulsions is one of the most important factors determining their suitability for applications in food and beverage industries (McClements 2015). In this experiment, we select the dependence of storage period upon curcumin contents and nanoemulsion droplet size to represent curcumin nanoemulsion storage stability.

Figure 4a, b illustrated the influence of the emulsifiers on the curcumin content in the nanoemulsions prepared by MCT and linseed oil during storage periods, respectively. Lecithin was found to lead to higher curcumin contents at the beginning of storage (Fig. 2). However, curcumin contents of such nanoemulsions showed more significant time dependence than those prepared with Tween-80, and the curcumin content gradually decreased to lower levels in lecithin emulsions than in the Tween-80 ones when they were stored for long time. In late storage time, the curcumin content of nanoemulsion prepared with Tween-80 is the almost the same as lecithin. Figure 4c, d showed the changes in the curcumin loaded nanoemulsion droplet size during 60-days storage. In the MCT nanoemulsions, the

droplet size was found to be independent of storage time when Tween-80 was used as emulsifiers. However, when lecithin was used, the droplet size decreased from 812.4 to 320.9 nm. Similar results were also found in curcumin nanoemulsions prepared with linseed oil as the oil phase. According to previous study, depletion effects would result in droplet flocculation (Iwanaga et al. 2008). In general, an emulsion is an essentially thermodynamically unstable system, which means standing for long time can lead to stratification. The presence of emulsifiers impedes small-size droplets from aggregating into larger ones, and such effects would protect curcumin from decomposition. Aggregation is therefore considered the reason for curcumin decomposition. This result indicated that Tween-80 emulsifiers prevented droplet growth and gravitational separation and therefore would produce stable nanoemulsions (Yang et al. 2013). In terms of Fig. 5a–d, we can conclude that changes in droplet size and curcumin contents are highly correlated.

Influence of storage temperature on curcumin nanoemulsion prepared with different emulsifiers was shown in Fig. 6. The influence of storage temperature on the nanoemulsions fabricated with Tween-80 was insignificant, which means such system exhibited good heat resistance and preferable storage stability. However, the influence of temperature on the nanoemulsions prepared with lecithin was significant. It could be concluded that in the lecithin nanoemulsions, the magnitude of decreases in curcumin content and particle size of nanoemulsions were significantly enhanced as the storage temperature increased. Oil droplets, stabilized by non-ionic surfactants, become unstable when they are heated close to the critical temperature (known as “phase transition temperature” or PIT), because of dehydration of surfactant polar head groups (Anton and Vandamme 2009). Meanwhile, nanoemulsion system is a thermodynamically unstable system. Temperature plays an important role in thermodynamic movement. With the increase of temperature, the molecular thermal motion rate increases. Nanoemulsion prepared by Tween-80 as emulsifier, compared with lecithin as emulsifier, is more stable to overcome the stratification phenomenon caused by molecular thermal movement after high pressure homogeneous due to a smaller molecular weight of emulsifier and the particle size of curcumin nanoemulsion (Israelachvili 2011).

Influence of different SOR

The surfactant-to-oil ratios (SOR) and oil concentration have an essential impact on curcumin loaded nanoemulsion characteristics. Similarly, as in the previous part, we discussed the influences of SOR on curcumin nanoemulsions

Fig. 4 Effect of characterization variation with storage time at different emulsifier types: **a** MCT as oil phase, curcumin content variation; **b** linseed oil as oil phase, curcumin content variation; **c** MCT as oil phase, size variation; **d** linseed oil as oil phase, size variation

from three aspects including characteristics, physical stability and storage stability.

Influence of SOR on characterization of curcumin nanoemulsion

Figure 2a–c shows the relationship between curcumin contents and oil concentrations of nanoemulsions. It is expected that curcumin content increased with increasing the oil content because curcumin may be over-saturated in each kind of emulsifier and oil phase. It is worth to mention that in different kinds of emulsifiers and oil phases, increasing the same proportion of oil concentration will lead to the increase of different proportion of curcumin content. This also confirmed the previous conclusion that

emulsifier plays an important part of curcumin loaded nanoemulsions. The increment is more significant in the system when Tween-80 as emulsifier and MCT as oil phase and lecithin as emulsifier, compared to the system with WPI as emulsifier and linseed oil as oil phase as well as the system with WPI as emulsifier and canola oil as oil phase. It can be concluded that oil concentration and SOR are essential part of curcumin nanoemulsion.

Figure 2d–f shows the relationship between SOR and droplet size of curcumin nanoemulsions. Except for those prepared with WPI as emulsifiers, the droplet size of nanoemulsions increased with smaller SOR. Moreover, when Tween-80 and lecithin were used as emulsifiers, the dependence of droplet size on SOR was less dramatic than that of the nanoemulsions with acacia as emulsifiers. It can

Fig. 5 Effect of content variation with storage time at different emulsifier concentrates: **a** tween-80 as emulsifier, MCT as oil phase; **b** lecithin as emulsifier, MCT as oil phase; **c** tween-80 as emulsifier, linseed oil as oil phase; **d** tween-80 as emulsifier, linseed oil as oil phase

be easy to conclude that the SOR plays an essential part on nanoemulsion droplet size. As expected, larger SOR values tended to result in smaller droplet size of the curcumin nanoemulsion, regardless of the emulsifier species. This result can be attributed to the following reasons. First of all, more absorbed surfactants would create lower interfacial tensions for emulsion droplets. Also, a higher SOR value leads to faster coverage of droplet surfaces by surfactant molecules, providing better protection against coalescence (Jafari et al. 2008).

Figure 2g–j shows the relationship between oil concentration and zeta-potential of curcumin nanoemulsions. When different types of emulsifiers were involved, different SOR dependences of zeta-potential were observed. In

most instances, except for those with WPI as emulsifiers, zeta-potentials of nanoemulsions were found to be independent of SOR. Combined with previous discussions, the emulsifier plays an essential role on curcumin nanoemulsions, and the droplets fabricated with Tween-80 and lecithin can obtain higher curcumin contents and smaller droplet sizes than the others.

The microstructures of various nanoemulsions are shown in Fig. 3, which verified previous conclusions that droplet size of nanoemulsions increased with decrease of SOR. Compared with Fig. 3d, it can be concluded from Fig. 3b that the high-SOR nanoemulsions displayed better monodispersity than those with low SOR values when Tween-80 was used as the surfactant. In the case of using

Fig. 6 Effect of MCT as oil phase characterization variation with storage time at different storage temperatures: **a** tween-80 as emulsifier, content variation; **b** lecithin as emulsifier, content variation; **c** tween-80 as emulsifier, size variation; **d** lecithin as emulsifier, size variation

lecithin as surfactant, the microstructures of the curcumin nanoemulsion droplets were substantially the same as those using Tween-80. However, it should be noticed that the decrease of SOR caused droplet aggregation. Meanwhile, there is an exception that aggregation did not occur in the nanoemulsions prepared by Tween-80 as emulsifier at lower SOR, where dispersing actually took place. This phenomenon was probably a result of emulsifier improving monodispersity of curcumin nanoemulsions. It has been found that decrease in SOR would result in extensive flocculation and coalescence of emulsion droplets (Yuan et al. 2013), and we drew the similar conclusion here. Reduced SOR values would generally deteriorate the uniformity and dispersion of the curcumin nanoemulsions.

Influence of SOR on physical stability of curcumin nanoemulsion

Effects of oil concentration on physical stability of curcumin nanoemulsions were also measured through their transmission changes. The emulsion was more stable if its transmission changed less frequently. The impacts from SOR on nanoemulsion physical stability are illustrated in Fig. 3e. Reduced SOR values were found to appropriately improve the physical stability of nanoemulsions. Theoretically, decreased SOR will cause extensive droplet aggregation, mainly due to kinds of reasons discussed previously such as bridging flocculation and charge neutralization (McClements et al. 1993). However, we can conclude from

the results that the decreased SOR can improve the ability of nanoemulsions to resist the effects of centrifugal forces. The main reason for this phenomenon is supposed to be the increased viscosity (Jumaa and Müller 1998). When the viscosity of an emulsion is increased, the Brownian motions of microscopic droplets are weakened, resulting lower aggregation effects due to thermal motions. Therefore, such emulsions perform better physical stability when subjected to centrifugal forces.

Influence of SOR on storage stability of curcumin nanoemulsion

To find the relationship between SOR and storage stability of nanoemulsions, the effects of various oil phase concentrations (5, 10, 15, 20, 25, 30 wt%), emulsifiers (Tween-80, lecithin, WPI, acacia) and oil species (MCT, canola oil, linseed oil) of curcumin nanoemulsions were evaluated in terms of the changes in curcumin contents and droplet size during a 60-day shelf storage.

The time dependence of curcumin contents of nanoemulsions prepared with MCT as the oil phase during storage was presented in Fig. 5a, b. When Tween-80 was used as emulsifiers, the nanoemulsions maintained fine storage stability regardless of SOR values. However, when lecithin was used, a negative correlation was observed between the decomposition rate of curcumin and SOR during storage. In the Fig. 5b, the result represents the slope of the curve, which increases as oil concentrate increases (Fig. 5b). In the case of the nanoemulsions with linseed oil as the oil phase, changes in the curcumin contents were not as significant as those with MCT as the oil phase and the result is basically consistent with MCT as oil phase (Fig. 5c, d). This phenomenon is attributed to Ostwald ripening where larger droplets grew at the expense of smaller droplets (Li et al. 2009). Ostwald ripening is due to the molecular diffusion through a continuous oil phase from the aqueous phase droplets.

Figure 6 illustrated the time dependence of droplet size of nanoemulsions prepared under different conditions during storage. The nanoemulsions with Tween-80 as emulsifiers were relatively stable, and no significant changes were found in droplet size. However, in other conditions, such as nanoemulsions with lecithin as emulsifiers, the droplet size is negatively correlated with the SOR value, indicating that the storage stability is positively correlated with SOR of curcumin nanoemulsions. Emulsion stability is a complex issue and can be determined by numerous factors such as droplet size, viscosity and environmental conditions. In general, smaller droplets have a slighter tendency of creaming whereas larger droplets were more inclined to aggregate because they are more susceptible to the influence of Brownian motions and therefore

have more chances to collide with each other (McClements 2015). This may explain the previous findings that the nanoemulsions containing smaller droplets were more stable (Kabalnov 2001). Another reason is that small droplets decrease the Ostwald ripening rate, which improves dispersion and the concentration of free surfactant (Kabalnov 2001; Kabalnov and Shchukin 1992).

Conclusion

In this study, effects of various emulsifiers and different SOR values on the preparation of curcumin nanoemulsions were investigated. In the aspect of the species of emulsifiers, on the one hand, the nanoemulsions fabricated with Tween-80 and lecithin possessed higher curcumin contents and smaller droplets, which was verified by the microstructures of the droplets. On the other hand, compared with Tween-80 and lecithin as the emulsifier, O/W system prepared by Tween-80 exhibited better physical stability and storage stability, which was confirmed by less transmission under high-speed centrifugation and less variation of curcumin content and particle size during a 60-day storage. In the aspect of SOR, the curcumin contents and droplet sizes were both negatively correlated with SOR values. The viscosity of curcumin nanoemulsions increased with the increased oil concentration. Nanoemulsion with lower SOR exhibited better physical stability detected by integrated transmission-time. Meanwhile, in the case of relatively stable nanoemulsions, such as those with Tween-80 as emulsifiers, the influence of SOR on storage stability was not significant. However, in the case of other emulsifiers, higher SOR will lead to higher curcumin content but larger droplet size, which means relatively poor storage stability. In summary, nanoemulsions with Tween-80 as emulsifiers and higher SOR values are optimized to be excellent carriers for curcumin due to the better storage stability and physical stability.

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Compliance with ethical standards

Conflict of interest The author declares that they no conflict of interest.

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