Effects of Small and Large Molecule Emulsifiers on the Characteristics of β-Carotene Nanoemulsions Prepared by High Pressure Homogenization

Like Mao[1], Duoxia Xu[1], Jia Yang[1], Fang Yuan[1], Yanxiang Gao[1]* and Jian Zhao[2]

[1]College of Food Science and Nutritional Engineering, China Agricultural University,
CN-100083 Beijing, PR China

[2]School of Chemical Sciences and Engineering, The University of New South Wales,
Sydney, NSW 2052, Australia

Summary

Oil-in-water (O/W) nanoemulsion allows the preparation of lipophilic nutrients such as carotenoids in liquid form, which may improve their bioavailability and broaden applications. In the present study, polyoxyethylene sorbitan monolaurate (Tween 20, TW-20), decaglycerol monolaurate (DML), octenyl succinate starch (OSS), whey protein isolate (WPI), and finally a blend of TW-20 and WPI (1 % by mass) have been tested for the preparation of β-carotene nanoemulsions. Interfacial tension, droplet size, zeta potential, microstructures observed by atomic force microscopy (AFM), stability of the emulsion and β-carotene have been compared. Results show that nanoemulsions stabilized with TW-20 and DML had smaller droplet size, but relatively poorer stability, compared with the ones stabilized with OSS and WPI (p<0.05). WPI was able to protect β-carotene effectively from degradation, whereas OSS was not when emulsions were stored at 55 °C for 12 days. When a blend of TW-20 and WPI was used, the stability of nanoemulsions was significantly improved (p<0.05). However, no significant effect of the mixed emulsifiers was found on the stability of β-carotene in the emulsions (p>0.05).

Key words: nanoemulsion, emulsifiers, β-carotene, droplet size, emulsion stability

Introduction

In the past decades, great attention has been paid to the carotenoids family, like β-carotene, lycopene, lutein, and astaxanthin, due to their unique antioxidant properties (*1,2*). However, carotenoids are insoluble in water and only slightly soluble in oil at room temperature, which greatly limits their applications. Furthermore, carotenoids from vegetables are probably bound in a protein complex or exist in crystal form, which may lead to poor bioavailability (*3*).

Great attempts have been made to improve bioavailability of carotenoids, and the emulsification has proved to be a good method. Carotenoids incorporated in oil droplets have much better bioavailability (*4,5*), and oil-in-water (O/W) emulsions offer excellent dispersion in water. Therefore, the emulsions containing carotenoids have attracted considerable attention. For example, Ax *et al.* (*6*) used high pressure homogenizer to prepare lycopene emulsions and evaluated the thermal stability of lycopene in emulsion systems. Ribeiro *et al.* (*7*) prepared astaxanthin emulsions by repeated premix membrane emulsification, and found the optimal ranges of pressure and dispersed phase ratio for the process, Losso *et al.* (*8*) optimized the parameters for preparing lutein-enriched O/W emulsions, and Batista *et al.* (*9*) investigated the rheological characteristics of lutein emulsions. Cheng *et al.* (*10*) studied the size distribution and size

stability of β-carotene submicron emulsions. Ribeiro *et al.* (11) subsequently compared different cellular uptake properties of lycopene and astaxanthin emulsions in colon carcinoma cell *in vitro*.

Recently, the interest in emulsion preparations has focused on submicron droplet size, and the emulsions have been termed as nanoemulsions. Nanoemulsions can be transparent or translucent (size range 50–200 nm) or 'milky' (up to 500 nm), and show kinetic stability during storage. Emulsions with nanosize droplets are suitable for efficient delivery of active ingredients through skin and enhance the penetration of ingredients, and they may be applied as substitutes for liposomes and vesicles (12). Nanoemulsions containing carotenoids (nanodispersions) prepared through an emulsification and evaporation technique were firstly reported by Tan and Nakajima (13,14). In our previous work, β-carotene nanoemulsions were also successfully prepared by high pressure homogenizer (15,16).

It is common knowledge that the emulsifier is an essential part of emulsion/nanoemulsion systems. Some of them are solely emulsifiers such as polysorbate and glycerol esters, which are defined as small molecule emulsifiers, while some have both emulsifying and stabilizing properties such as milk proteins and modified starches, which are defined as large molecule emulsifiers (or surface-active polymers). Of the carotenoid emulsions mentioned above, little attention has been given to the effects of emulsifiers on the properties of the emulsions. While Tan and Nakajima (13) and Yuan *et al.* (15) investigated the effect of small molecule emulsifiers, polyglycerol esters and polysorbate on stabilizing nanoemulsions. Large molecule emulsifiers, which are common ingredients in food formulations and usually coexist with the small molecule ones, have received little study.

Therefore, the purpose of the current study is to prepare β-carotene nanoemulsions with small and large molecule emulsifiers, as well as their blends, and to evaluate their effects on the formation and stabilization of the nanoemulsions. For small molecule emulsifiers, TW-20 and DML were preferred, as both of them had been proven to have the best emulsifying properties among the polysorbate and polyglycerol emulsifiers (13, 15). WPI and OSS were applied as large molecule emulsifiers, since they are very common food ingredients and well studied in the model system of O/W emulsions (17,18).

Materials and Methods

Materials

β-Carotene suspension (30 % by mass, of β-carotene in sunflower oil) was purchased from Xinchang Pharmaceutical Co., Ltd. (Zhejiang, PR China). Standard β-carotene (>95 % purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Medium chain triglyceride (MCT) oil was obtained from Lonza Inc. (Allendale, NJ, USA). Polyoxyethylene sorbitan monolaurate (Tween 20, TW-20) was provided by Fucheng Chemical Reagents Factory (Tianjin, PR China). Decaglycerol monolaurate (DML, ML750) was supplied by Sakamoto Yakuhin Kogyo (Osaka, Japan). Whey protein isolate (WPI, BiPro) was

obtained from Davisco Food International (Le Sueur, MN, USA). Octenyl succinate starch (OSS, Purity Gum 2000) was kindly offered by National Starch and Chemicals (Shanghai, PR China). All other chemicals used were of analytical grade, unless otherwise stated.

Surface tension and interfacial tension analysis

Surface tension and interfacial tension were measured using the pendant drop method with the DataPhysics OCA20 contact angle system (DataPhysics Instruments, Germany). A drop of the emulsifier solution (1 % by mass) to be analyzed was formed at the bottom of a capillary column using the manual dosing system. The surrounding medium was the atmosphere for surface tension determination or MCT oil for interfacial tension determination. Charged Coupled Device (CCD) camera photographed the contour of the drop, from which the tension values were calculated using the SCA22 software automatically.

Preparation of β-carotene nanoemulsions

Oil-in-water nanoemulsions were prepared with MCT oil containing β-carotene (0.03 % by mass, in the final emulsion) as the dispersed phase and aqueous emulsifier solutions (1 % by mass, in the final emulsion) as the continuous phase. The emulsifier solutions (emulsifiers dissolved in 0.05 M phosphate buffer, pH=7.0) were kept overnight to ensure complete dispersion and dissolution of the emulsifiers, and sodium azide (0.01 % by mass) was added as an antimicrobial agent. β-Carotene was first dissolved in MCT oil at 140 °C for several seconds and then mixed with emulsifier solutions at 5000 rpm in a blender to form coarse emulsions, which were further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at different pressures. Nanoemulsions from the exit of the homogenizer were immediately cooled down to room temperature and sampled to measure the droplet size, zeta potential, microscopic image and stability. For storage tests (at 55 °C for 12 days), the nanoemulsions were kept in screw-capped amber bottles and flushed with nitrogen. Nanoemulsions were first prepared with sole emulsifiers, and then a designed series of blends of TW-20 and WPI (TW-20/WPI=1:4, 1:1 or 4:1, the overall concentration of the mixed emulsifiers was 1 % by mass) were used to investigate the interaction of the two emulsifiers.

Determination of droplet size, size distribution and zeta potential

Droplet size, size distribution and zeta potential of β-carotene nanoemulsions were determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed detector angle of 90°. Nanoemulsions were diluted to minimize multiple scattering effects prior to each measurement. Results were described as size in nm for droplet size, polydispersity index (PDI) for size distribution, and zeta potential (mV).

Evaluation of nanoemulsion stability

Nanoemulsion stability (the aggregation tendency of droplets in the emulsions) was evaluated using a Tur-

biscan instrument (Formulaction, L'Union, France). The principle underlying the operation of this instrument was detailed by Lemarchand *et al.* (*19*). The emulsion sample was transferred to a cylindrical glass cell and analyzed by a light beam emitted in near infrared (880 nm) wavelength which scanned the sample cell from the bottom to the top. Two synchronous optical sensors received respectively the light transmitted through the sample and the light backscattered by the sample. By scanning the sample at preset intervals, a pattern of the light flux (transmission or backscattering) as a function of the sample height was obtained, giving a macroscopic fingerprint of the sample at a given time, so that the stability of the emulsion could be evaluated. In this study, the sample in the cell was scanned every 30 min for 12 h at 55 °C. Here stability index was used to represent the stability of the emulsions and it was calculated from the changes in backscattering flux during the period of 12 hours. The value of stability index was the reciprocal of delta backscattering per hour ($\Delta BS/t$).

Analysis of β-carotene content

β-Carotene content in the nanoemulsion was determined by high-performance liquid chromatography (HPLC) using an Agilent 1100 HPLC instrument equipped with a diode array detector. β-Carotene was firstly extracted with ethanol and *n*-hexane from the nanoemulsions (*6*), and then the *n*-hexane solutions were diluted to an appropriate concentration with acetone. Samples were separated on a Diamonsil C18 column (250×4.6 mm, i.d. 5 µm; Agilent, USA) with the elution system of 95 % acetone and 5 % purified water. The flow rate was 1 mL/min and the injection volume was 10 µL. Peak areas were recorded by the diode array detector set at 450 nm (*20*). The concentration of β-carotene in the samples was obtained by referring to a standard curve of β-carotene (linear range from 1.00 to 8.00 mg/L, R^2=0.9993).

Atomic force microscopic (AFM) observation

β-Carotene nanoemulsions were also observed by AFM (Agilent 5500 AFM, USA) for microstructure. Diluted samples were deposited on freshly cleaved mica substrates, and dried in the air. Different regions of the mica were scanned by AFM with the Si_3N_4 probe, applying the Acoustical Alternating Current (AAC) tapping mode. In order to get more information, both the plane image and the three-dimensional image of the surface morphology were presented.

Statistical analysis

The whole experiments were conducted in duplicate and all measurements were done at least in triplicate. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 12.0 package. Significant differences of means ($p<0.05$) were determined by Duncan's multiple range test.

Results and Discussion

Effect of different emulsifiers on the droplet size of the nanoemulsions

Table 1 shows the droplet parameters of the β-carotene nanoemulsions prepared at various homogenization pressures with different emulsifiers. The mean diameters of the droplets ranged from 115.0 to 303.0 nm, with the PDI range of 0.09–0.30, which indicates ideal distributions of the droplets. In general, droplet sizes were smaller in nanoemulsions prepared with TW-20 and DML, than those with WPI and OSS ($p<0.05$). For example, at a homogenization pressure of 80 MPa, the nanoemulsions emulsified with TW-20 and DML showed similar droplet size of 132 nm, and the droplet sizes of those stabilized with WPI and OSS were 183 and 212 nm, respectively. Interfacial behaviour of the four emulsifiers may contribute to the difference of droplet sizes. Table 2 represents the surface tensions of the emulsifier solutions and interfacial tension of the oil and the emulsifier solutions. As indicated, TW-20 and DML dissolved in water had much smaller surface tension values than water, and showed low interfacial tension when they were mixed with oil, which was favourable for the formation of small droplets. In contrast, WPI and OSS had poor surface activity, and their large molecule structure would prevent close packing of the points of contact with the interface, resulting in relatively high interfacial tension (*21*). Additionally, large molecule emulsifiers possessed low adsorption kinetics (*22*), and the droplet breakup occurred at a very short period in the homogenizer. Therefore, the emulsifiers could not efficiently and quickly stabilize the new droplets, leading to coalescence of the droplets. For the two large molecule emulsifiers, the nanoemulsions with smaller droplet sizes were generated from WPI than OSS ($p<0.05$) in the pressure range of homogenization. This could be explained by a possible conformation change of WPI occurring during homogenization and afterwards, as the protein chain might unfold during adsorption, resulting in the expo-

Table 1. Droplet sizes and size distributions of β-carotene nanoemulsions

Emulsifiers	20 MPa		80 MPa		140 MPa	
	Size/nm	PDI	Size/nm	PDI	Size/nm	PDI
DML	203.7±7.6	0.16±0.04	132.0±2.5	0.20±0.01	115.0±1.7	0.23±0.01
TW-20	195.8±3.1	0.13±0.03	132.5±2.9	0.21±0.03	117.2±4.3	0.30±0.04
WPI	224.8±7.3	0.17±0.05	183.3±3.6	0.12±0.02	160.3±1.4	0.12±0.03
OSS	303.0±5.3	0.13±0.04	212.2±2.3	0.14±0.06	178.5±1.9	0.09±0.04

Size, cumulative mean diameter of the β-carotene nanoemulsions; PDI, polydispersity index; nanoemulsions were prepared with four emulsifiers (1 % by mass) under different homogenization pressures at 55 °C (mean±SD, *N*=6)

Table 2. Surface tensions of different emulsifier solutions, and interfacial tensions of the emulsifier solutions and oil

	Water	TW-20 solution	DML solution	OSS solution	WPI solution
Surface tension/(mN/m)	72.46±0.01	38.80±0.14	38.40±0.10	71.60±0.08	62.20±0.06
Interfacial tension/(mN/m)	22.62±0.03	4.59±0.05	8.94±0.08	20.64±0.10	16.88±0.13

All the measurements were made at room temperature; interfacial tensions were measured against MCT oil

sure of more hydrophobic groups, which would facilitate emulsification (*23*). Although OSS would follow similar behaviour like proteins, the newly exposed hydrophobic sites would be much smaller due to their fewer surface active groups, mainly octenyl succinic acid chains (*24*). However, when the homogenization pressure increased up to 80 MPa, the difference in the droplet size became much smaller. A possible explanation is that protein-protein interactions formed between neighbouring droplets through hydrophobic attraction reduced the emulsification efficiency.

Fig. 1 shows two representative AFM images of β-carotene nanoemulsions, one stabilized by TW-20, and the other by WPI. Careful examination of the AFM images revealed that most of the droplets exhibited approximately spherical morphology with mean diameters of 100–200 nm, which were similar to those obtained by dynamic light scattering analysis. The droplets were like solid objects, whose surfaces were mostly rugged with sags and crests. Furthermore, some droplets aggregated, especially in the nanoemulsion stabilized with TW-20, which demonstrated the instability of the emulsion. Comparatively, droplets in the WPI-stabilized nanoemulsion were better distributed, although proteins on the neighbouring droplets might be in contact with each other.
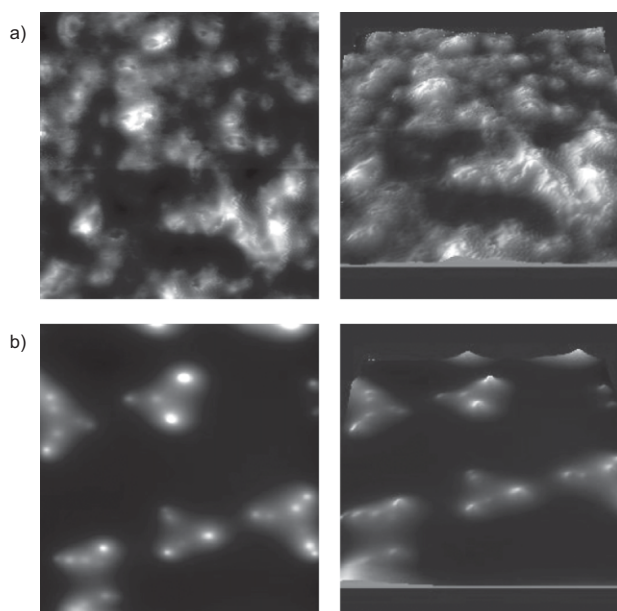
Effects of different emulsifiers on the stability of β-carotene nanoemulsions

As the nanoemulsion incorporated β-carotene, its stability included the stability of the nanoemulsion and the stability of β-carotene. Fig. 2 illustrates the result of Turbiscan analysis, which showed that the nanoemulsion stabilized by OSS had higher stability index values, meaning greater stability, followed by those stabilized by WPI and TW-20, while DML-stabilized nanoemulsion had the poorest stability. OSS and WPI are large molecule emulsifiers, and they can form mechanically strong interfacial layers and cause steric hindrance to prevent droplet coalescence. Moreover, droplets in WPI emulsions often carry a large number of ions (with a zeta potential of −(17.3±1.06) mV) and OSS solution showed a relatively high viscosity, which might improve their stabilizing properties. Small molecule emulsifiers stabilize emulsions mainly based on the Marangoni mechanism, and generally do not give highly cohesive or viscous surface layers (*21*), and droplets in the emulsions have poor ion-loading ability (zeta potentials of TW-20 and DML-stabilized nanoemulsions were −(5.13±0.45) mV and −(5.21±0.64) mV, respectively). These two factors probably led to the relatively poorer stability of TW-20 and DML stabilized nanoemulsions.

Due to its highly unsaturated structure (11 double bonds, 9 of which are unconjugated), β-carotene is considered to be very sensitive to thermal and oxidative degradation during processing and storage. In emulsion
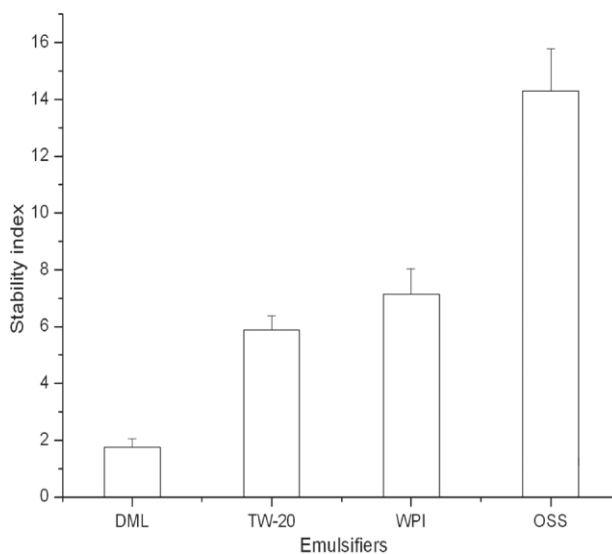

Fig. 1. Representative AFM images of β-carotene nanoemulsions (prepared at 80 MPa and 55 °C): a) TW-20-stabilized nanoemulsion at the scan area of 7.5×7.5 μm² (the left one is a plain image and the right one is the corresponding three-dimensional image); b) WPI-stabilized nanoemulsion at the scan area of 10×10 μm² (the left one is a plain image and the right one is the corresponding three-dimensional image)

Fig. 2. Stability of β-carotene nanoemulsions prepared with different emulsifiers. Turbiscan measurements were performed every 30 min for 12 h at 55 °C. Nanoemulsions were prepared at 80 MPa and 55 °C

systems, the degradation of β-carotene during storage might be greatly accelerated by two factors: large surface area of the oil droplets as a result of size reduction to nanometer range and possible formation of free radicals during high pressure homogenization process (*14*). In the present work, the stability of β-carotene was also found to be strongly influenced by the emulsifiers used (Fig. 3). The most severe degradation of β-carotene was detected in the OSS-stabilized emulsion, where only about 23 % of the original β-carotene was left at 55 °C after 12 days. The least severe degradation occurred in WPI-stabilized emulsion with approx. 72 % of the β-carotene retained. The major constituents of WPI are β-lactoglobulin and α-lactalbumin, and both of them contain cysteyl residues, disulphide bonds and thiol functional groups, which can inhibit lipid oxidation by scavenging free radicals (*25*). Therefore, WPI can play the role of an antioxidant, which probably resulted in the less severe degradation of β-carotene in the WPI-stabilized emulsion. The severe β-carotene degradation that occurred in the OSS-stabilized emulsion was probably a result of the loose interfacial layer on the oil droplets, although OSS-stabilized emulsion had higher stability index values. As discussed above, OSS was a poorly adsorbed large molecule emulsifier, with the adsorbed molecules being loosely arrayed, and the interaction between molecules was quite weak (*21*). When the emulsifier concentration was lowered to 1 % by mass, the amount of emulsifiers on the interface was small and it could not form a compact layer to protect β-carotene very well.
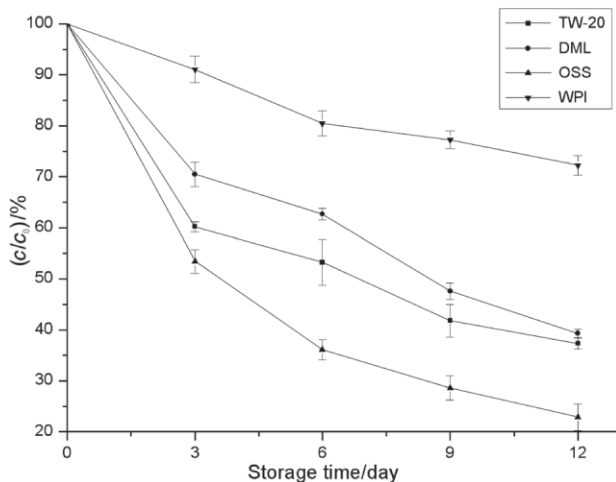

Fig. 3. Degradation of β-carotene in the nanoemulsions prepared with different emulsifiers when stored at 55 °C for 12 days. c is β-carotene concentration in nanoemulsions at the time of sampling, c_0 is initial β-carotene concentration in nanoemulsions

Regarding β-carotene nanoemulsions stabilized with TW-20 and DML, β-carotene was a little more stable in DML-stabilized emulsion than in the one stabilized with TW-20, and 37–39 % of the original β-carotene was kept after storage. Similar results were obtained by Tan and Nakajima (*13,14*), where 38.1 % of β-carotene were retained in DML-stabilized emulsion and 25–56 % in the emulsions stabilized with TW-20. The present evaluation

method was an accelerated test, while in actual applications, emulsions should be stored at lower temperature and the addition of antioxidants is required.

Physicochemical properties of the β-carotene nanoemulsion stabilized by a blend of TW-20 and WPI

Many studies have indicated that nonionic surfactants such as Tween might influence the physicochemical properties of a protein-stabilized emulsion in a number of ways, such as competitive adsorption, surfactant-protein interactions, and depletion flocculation (*26,27*). It is a well established fact that the existence of surfactant is likely to change the conformation of protein, leading to different emulsifying properties, changeable sensitivity to heat, pH, and ion strength of the protein (*28*). To evaluate such effect, comparisons between the emulsifying properties of the TW-20 and WPI blend at different ratios were conducted, and emulsions with the sole emulsifiers served as controls.

McClements (*29*) concluded that in emulsions stabilized with TW-20, 8 % by mass, emulsifier was required in the aqueous phase to induce depletion flocculation. Therefore, depletion flocculation would not have significant influence on our research system, since the concentration of TW-20 was relatively low in the present study. Measurements of the droplet size showed that an increase in WPI concentration was accompanied by an elevation in droplet size, and the increment was quite insignificant when WPI proportion was below 50 % (Table 3). TW-20 is more easily adsorbed than WPI, and WPI can be displaced by TW-20 from the interface (*21*). Therefore, when WPI percentage was low, emulsifiers on the interface were dominated mostly by TW-20, and the adsorbed TW-20 was sufficient to cover all the surface of the droplets, which resulted in small changes of droplet size. However, when TW-20/WPI ratio reached 1:4, although some protein was displaced by TW-20, WPI was still able to dominate the interface, and the droplets became bigger. Though the blending ratios of the two emulsifiers were different, droplets in all these emulsions were smaller than those in the emulsions stabilized by WPI only. Similar results were obtained by Jafari *et al.* (*24*), who prepared nanoemulsions using OSS with or without TW-20 and found that the droplet size was much smaller in the emulsions stabilized with mixed emulsifiers. The surface tension and interfacial tension did not change significantly when WPI percentage ranged from 0 to 80 % in the mixed emulsifiers (Table 3), which might be related to the interaction of the two emulsifiers. When a nonionic surfactant is added to the protein-stabilized emulsion, interactions might occur between the nonpolar tail of the surfactant molecule and the exposed hydrophobic patches on the protein molecule (*26,30*). As Tween actually binds to the protein, its conformation might change and it becomes more surface active (*21*).

Fig. 4 demonstrates that stability index of the emulsions increased from 5.88 to 20.00 and then was reduced to 7.14 when WPI percentage increased from 0 to 100 %. It apparently showed that emulsions stabilized by mixtures of TW-20 and WPI were more stable, compared

Table 3. Properties of β-carotene nanoemulsions stabilized with mixed TW-20 and WPI (1 % by mass) emulsifiers at different ratios or sole emulsifiers

	TW-20	TW-20/WPI=4:1	TW-20/WPI=1:1	TW-20/WPI=1:4	WPI
Size/nm	132.50±2.90	138.00±2.20	140.00±2.30	166.70±1.50	183.30±3.60
PDI	0.21±0.03	0.18±0.02	0.17±0.03	0.16±0.01	0.12±0.02
Zeta potential/mV	−5.13±0.45	−5.43±0.45	−7.14±0.79	−8.99±0.52	−17.30±1.06
Surface tension/(mN/m)	38.8±0.14	35.32±0.03	40.34±0.01	39.35±0.04	62.20±0.06
Interfacial tension/(mN/m)	4.59±0.05	5.01±0.02	5.18±0.02	5.13±0.01	16.88±0.13

All the measurements were made at room temperature; interfacial tensions were measured against MCT oil; the emulsions were prepared at 80 MPa and 55 °C
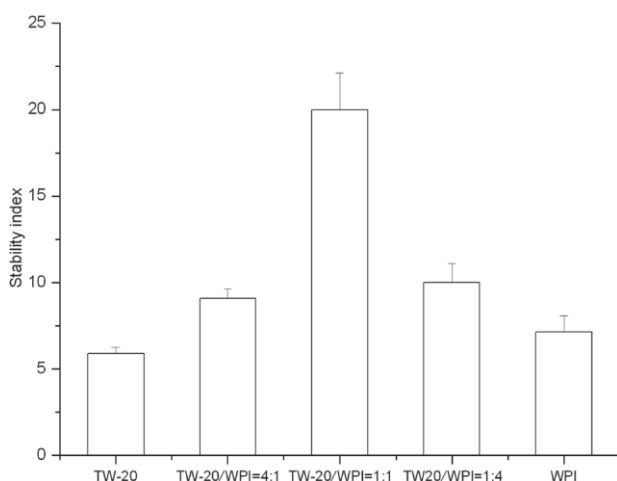

Fig. 4. Stability of β-carotene nanoemulsions prepared with TW-20 and WPI mixed emulsifiers at different ratios. Turbiscan measurements were performed every 30 min for 12 h at 55 °C. Nanoemulsions were prepared at 80 MPa and 55 °C

with those stabilized by either TW-20 or WPI, and the emulsion became the most stable when the ratio of the two emulsifiers was 1:1. As discussed above, protein was likely to be displaced by small molecule emulsifiers, but some studies indicate that when protein displacement occurs, the small molecule emulsifier pushes the protein aside from parts of the interface rather than displaces the protein molecule one by one. The protein molecule does not leave the surface, but forms multilayers over restricted areas of the interface (*31,32*). Zeta potential measurement showed that the intensity of the charged ions increased as WPI gradually dominated the mixed emulsifiers (Table 3), which was just as expected. The aforementioned two points might help explain the improved stability of the nanoemulsions stabilized with the mixed emulsifiers. However, it was not sufficient to explain the different stabilizing properties of the mixed emulsifiers. The actual underlying mechanism was not clear, and the interaction between the two emulsifiers might account for the phenomena to some extent. Wilde and Clarke (*30*) stated that TW-20 formed 1:1 complex with β-lactoglobulin at the interface. This was probably the reason why the nanoemulsion prepared with TW-20/WPI mixed emulsifiers at 1:1 ratio had the best stability. When TW-20/WPI ratio was 4:1 or 1:4, the interaction was able to exist as well, but it was relatively weak.

In terms of the stability of β-carotene, it was not clear whether the blend of the two emulsifiers had positive effects. About 57.22, 63.35, and 55.54 % of the original β-carotene were retained after 12 days of storage in the mixed emulsions at the ratio of 4:1, 1:1 and 1:4, respectively (Fig. 5). The β-carotene degradation rates were just between those in the nanoemulsions stabilized by WPI and TW-20 alone. Theoretically, much more β-carotene should be retained as a result of the increase in WPI concentration. However, for the three mixed emulsifier systems, degradation rates of β-carotene were rather close to each other, and a relatively higher proportion of β-carotene was retained when the mixed ratio was 1:1.
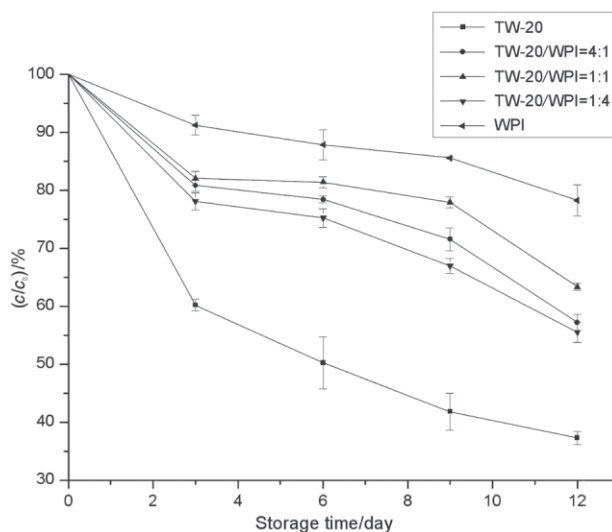

Fig. 5. Degradation of β-carotene in the nanoemulsions prepared with TW-20 and WPI mixed emulsifiers at different ratios when stored at 55 °C for 12 days. c is β-carotene concentration in nanoemulsions at the time of sampling, c_0 is initial β-carotene concentration in nanoemulsions

Conclusions

TW-20 and DML were able to reduce oil-water interfacial tension and form nanoemulsions with much smaller droplets, but the droplets in these emulsions were easily aggregated. In contrast, OSS- and WPI-stabilized emulsions had bigger droplets, but they were rather stable because of the strong interfacial layers. β-Carotene in WPI-stabilized emulsions was quite stable, but it de-

graded quickly in the OSS-stabilized one. A blend of TW-20 and WPI was found to improve the stability of β-carotene nanoemulsion, especially when TW-20/WPI ratio was 1:1, probably as a result of the surfactant-protein interaction.

Acknowledgements

References

1. H. Sies, W. Stahl, A.R. Sundquist, Antioxidant functions of vitamins. Vitamins E and C, β-carotene, and other carotenoids, *Ann. NY Acad. Sci. 699* (1992) 7–20.

2. S. Kiokias, M.H. Gordon, Antioxidant properties of carotenoids *in vitro* and *in vivo*, *Food Rev. Int. 20* (2004) 99–121.

3. A.W. Williams, T.W. Boileau, J.W. Erdman Jr., Factors influencing the uptake and absorption of carotenoids, *Proc. Soc. Exp. Biol. Med. 218* (1998) 106–108.

4. C.E. West, J.J.M. Castenmiller, Quantification of the 'SLAMENGHI' factors for carotenoid bioavailability and bioconversion, *Int. J. Vit. Nutr. Res. 68* (1998) 371–377.

5. R.S. Parker, Bioavailability of carotenoids, *Eur. J. Clin. Nutr. 51* (Suppl. 1) (1997) 86–90.

6. K. Ax, E. Mayer-Miebach, B. Link, H. Schuchmann, H. Schubert, Stability of lycopene in oil-in-water emulsions, *Eng. Life Sci. 3* (2003) 199–201.

7. H.S. Ribeiro, L.G. Rico, G.G. Badolato, H. Schubert, Production of O/W emulsions containing astaxanthin by repeated premix membrane emulsification, *J. Food Sci. 70* (2005) E117-E123.

8. J.N. Losso, A. Khachatryan, M. Ogawa, J.S. Godber, F. Shih, Random centroid optimization of phosphatidylglycerol stabilized lutein-enriched oil-in-water emulsions at acidic pH, *Food Chem. 92* (2005) 737–744.

9. A.P. Batista, A. Raymundo, I. Sousa, J. Empis, Rheological characterization of coloured oil-in-water food emulsions with lutein and phycocyanin added to the oil and aqueous phases, *Food Hydrocolloids, 20* (2006) 44–52.

10. J. Cheng, D. Wang, S. Pan, Study of the particle distribution and stability of submicron β-carotene emulsion, *Food Sci. 27* (2006) 57–59 (in Chinese).

11. H.S. Ribeiro, J.M.M. Guerrero, K. Briviba, G. Rechkemmer, H.P. Schuchmann, H. Schubert, Cellular uptake of carotenoid-loaded oil-in-water emulsions in colon carcinoma cells *in vitro*, *J. Agric. Food Chem. 54* (2006) 9366–9369.

12. T. Tadros, P. Izquierdo, J. Esquena, C. Solans, Formation and stability of nano-emulsions, *Adv. Colloid Interface. Sci. 108–109* (2004) 303–318.

13. C.P. Tan, M. Nakajima, Effect of polyglycerol esters of fatty acids on physicochemical properties and stability of β-carotene nanodispersions prepared by emulsification/evaporation method, *J. Sci. Food Agric. 85* (2005) 121–126.

14. C.P. Tan, M. Nakajima, β-Carotene nanodispersions: Preparation, characterization and stability evaluation, *Food Chem. 92* (2005) 661–671.

15. Y. Yuan, Y. Gao, J. Zhao, L. Mao, Characterization and stability evaluation of β-carotene nanoemulsions prepared by

16. Y. Yuan, Y. Gao, L. Mao, J. Zhao, Optimisation of conditions for the preparation of β-carotene nanoemulsions using response surface methodology, *Food Chem. 107* (2008) 1300–1306.

17. E. Onsaard, M. Vittayanont, S. Srigam, D.J. McClements, Comparison of properties of oil-in-water emulsions stabilized by coconut cream proteins with those stabilized by whey protein isolate, *Food Res. Int. 39* (2006) 78–86.

18. A. Viswanathan, Effect of degree of substitution of octenyl succinate starch on the emulsification activity on different oil phase, *J. Polym. Environ. 7* (1999) 191–196.

19. C. Lemarchand, P. Couvreur, C. Vauthier, D. Costantini, R. Gref, Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan, *Int. J. Pharm. 254* (2003) 77–82.

20. M.I. Mínguez-Mosquera, D. Hornero-Méndez, Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reversed-phase HPLC, *J. Agric. Food Chem. 41* (1993) 1616–1620.

21. D.G. Dalgleish: Food Emulsifiers: Their Structures and Properties. In: *Food Emulsions*, S.E. Friberg, K. Larsson, J. Sjöblom (Eds.), Marcel Dekker Inc., New York, USA (2004) pp. 1–43.

22. S. Tesch, C. Gerhards, H. Schubert, Stabilization of emulsions by OSA starches, *J. Food Eng. 54* (2002) 167–174.

23. D.G. Dalgleish, Adsorption of protein and the stability of emulsions, *Trends Food Sci. Technol. 8* (1997) 1–6.

24. S.M. Jafari, Y. He, B. Bhandari, Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by high energy emulsification techniques, *Food Res. Int. 40* (2007) 862–873.

25. C. Sun, S. Gunasekaran, M.P. Richards, Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions, *Food Hydrocolloids, 21* (2007) 555–564.

26. T. Nylander: Interactions Between Proteins and Polar Lipids. In: *Food Emulsions*, S.E. Friberg, K. Larsson, J. Sjöblom (Eds.), Marcel Dekker Inc., New York, USA (2004) pp. 107–173.

27. D.J. McClements: *Food Emulsions: Principles, Practice and Techniques,* CRC Press, Boca Raton, FL, USA (1998).

28. K. Demetriades, D.J. McClements, Influence of pH and heating on physicochemical properties of whey protein--stabilized emulsions containing a nonionic surfactant, *J. Agric. Food Chem. 46* (1998) 3936–3942.

29. D.J. McClements, Ultrasonic determination of depletion flocculation in oil-in-water emulsions containing a non-ionic surfactant, *Colloid. Surf. A, 90* (1994) 25–35.

30. P.J. Wilde, D.C. Clark, The competitive displacement of β-lactoglobulin by Tween 20 from oil-water and air-water interfaces, *J. Colloid Interf. Sci. 155* (1993) 48–54.

31. A.P. Gunning, A.R. Mackie, A.R. Kirby, V.J. Morris, Scanning near field optical microscopy of phase separated regions in a mixed interfacial protein (BSA)-surfactant (Tween 20) film, *Langmuir, 17* (2001) 2013–2018.

32. A.R. Mackie, A.P. Gunning, M. Ridout, P. Wilde, V.J. Morris, Orogenic displacement in mixed β-lactoglobulin/β-casein films at the air/water interface, *Langmuir, 17* (2001) 6593–6598.

high pressure homogenization under various emulsifying conditions, *Food Res. Int. 41* (2008) 61–68.