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Effects of Surfactants on the Formation and Stability of Capsaicin-loaded Nanoemulsions

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Abstract: Food nanoemulsion systems consisting of water and oleoresin capsicum (OC), polyoxythylene sorbitan esters (Tween 20, 40, 60, and 80), propylene glycol (PG), sucrose monostearate (SM), and their corresponding mixtures were formulated to use as food vehicles. Tween 80 produced OC nanoemulsions with stable dispersions as one-phase systems, and the dertermined emulsification efficiencies clearly distinguished the ability of the various surfactants to emulsify OC. The nanoemulsions were prepared by both ultrasonication and self-assembly, and the nanoemulsion areas were determined using phase diagrams by measuring the sizes of the emulsions. One-phase nanoemulsions were presented, with a multiple cloudy region and phase separation that were dependent on the particle size of the emulsion. The OC nanoemulsions prepared by ultrasonication using systems of OC/Tween 80/water, OC/Tween 80/water+PG, and OC/Tween 80/water+SM, resulted in particle sizes ranging from 15 to 100 nm. Finally, the nanoemulsions maintained their initial sizes during storage, ranging from 65 to 92 nm.

Keywords: food nanoemulsion, self-assembly, surfactant, bioactive delivery system, oleoresin capsicum

Introduction

Capsaicin (the main pungent ingredient in hot pepper) is known as a useful functional ingredient because it can prevent oxidative stress-mediated chronic diseases such as cancer, cardiovascular disease, hypertension, stroke, and neurodegenerative disorders, due to its antioxidant activity in the absence of toxicity (1). Capsaicin is widely consumed as a spice and food additive and is utilized in medical applications (2-4). Capsaicin (*N*-[4-hydroxy-3-methoxy-phenyl]methyl-8-methyl-non-6-enamide) and dihydrocapsaicin (*N*-[4-hydroxy-3-methoxy-phenyl]methyl-8-methyl-non-6-nonanamide) are two members of the family of naturally occurring capsaicinoids, which make up the pungent components of hot pepper (5). Oleoresin capsicum (OC) is the industrial extraction of the dried ripe fruits of capsicums and contains a complex mixture of essential oils, waxes, colored materials, and several capsaicinoids.

One of the most promising nanotechnologies is the nanoemulsion delivery system, which offers the potential to improve the solubility and bioavailability of many bioactive ingredients such as carotenoids, polyunsaturated fatty acids, phytosterols, antioxidants, vitamin, minerals, and other natural compounds (6,7). Nanoemulsions are thermodynamically stable and transparent dispersions of oil and water stabilized by an interfacial film of surfactant molecules having droplet sizes less than 100 nm (8). The stability of a nanoemulsion depends on the preparation method and order of component additions (9). However, they have been very limited in their industrial applications, and there are only a few publications that explore the use of this technology for preparing carotenoid emulsions (10,11). High-energy emulsification methods such as high-shear stirring, high-pressure homogenization, and ultrasonic homogenization can be used to prepare nanoemulsions. Moreover, low-energy emulsification methods (12) have been developed by taking advantage of phase behavior and properties to promote the formation of nano-sized particles. These low energy techniques include self-assembly emulsification (13-15), spontaneous emulsification, phase transition (16,17), and phase inversion temperature methods (18-21).

Self-assembly emulsification can be accomplished with an isotropic mixture of oil, surfactant, and co-surfactant. These form fine oil-in-water (O/W) emulsions when introduced into aqueous media under agitation. The self-assembled structures that are formed in the oil phase at a certain dilution composition can be hydrated with water and form bicontinuous structures that eventually, upon further dilution, will invert into an O/W structure (22). Very few examples exist as systems based on food-grade surfactants such as ethoxylated sorbitan esters (Tweens) and sugar esters. Of all the nonionic surfactants, Tweens are especially attractive, commercially inexpensive surfactants that are used in food, cosmetic, and pharmaceutical applications. Food-grade O/W microemulsions can also be formulated using suitable nonionic surfactants and by additions of polyols and short-chain alcohols (23). It was found that additions of short-chain alcohols (such as ethanol) and polyols (glycerol or propylene glycol) helped the formation of both W/O and O/W microemulsions.

Recently, food nanotechnology has been particularly focused on nanoencapsulation for biofunctional ingredients

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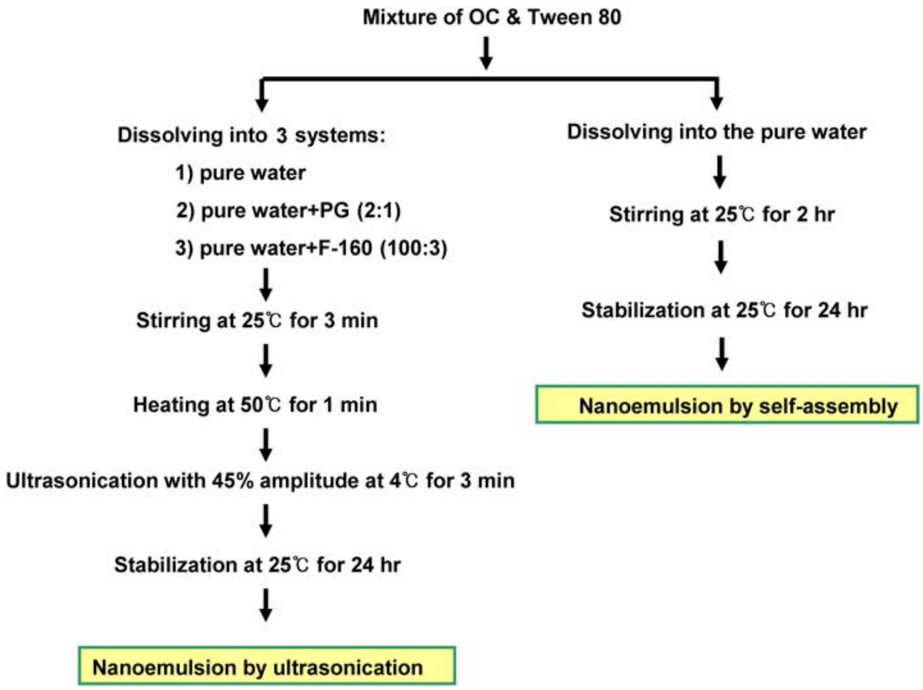

Fig. 1. Preparation of nanoemulsions by ultrasonication and self-assembly. OC, oleoresin capsicum; PG, propylene glycol; SM, sucrose monostearate.

such as capsaicin and resveratrol, and successful applications of nanoemulsions have been reported (24,25). A bioactive ingredient can be encapsulated in nanometer-sized structures, which are transparent systems of 20-500 nm in size and provide functionalities such as solubility, stability, and bioavailability (26). In this research, O/W nanoemulsions containing oleoresin capsicum were prepared by ultrasonication and self-assembly using different types of surfactants such as polyoxyethylene sorbitan esters of fatty acids (Tween 20, 40, 60, and 80), sucrose monostearate, and polypropylene glycol. The effects of the surfactant types and concentrations on the formation of the nanoemulsions were investigated by ternary phase diagrams. The stability of the nanoemulsions was evaluated by measuring the particle sizes of the emulsions during storage.

Materials and Methods

Materials Oleoresin capsicum (OC) was supplied from G&F (Seoul, Korea). The capsaicinoids content of the OC was 5.48±0.06 mg/g and its hotness degree was 100,000 SHU. Polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60), and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sucrose monostearate (SM; commercially available as F-160) was supplied by Ilshin Wells (Seoul, Korea), and polypropylene glycol (PG) was purchased from Sigma-Aldrich. Capsaicinoids for high performance liquid chromatography (HPLC) standards were purchased from Fluka (capsaicin:dihydrocapsaicin=65:35, Buchs SG, Switzerland). All other reagents were of analytical grade. Deionized distilled water was purchased from Waters (Milford, MA, USA).

Capsaicinoid content of OC The amounts of capsaicinoids in the oleoresin capsicum were quantified by HPLC using a modified procedure of Davis *et al*. (27). The HPLC system consisted of a pump (Gradient controller 601; Ever Seiko Corporation, Tokyo, Japan), a UV/VIS detector (S-3740; Soma Optics Ltd., Tokyo, Japan), and a data station (MultiChro^{TM}; Yullin Technology, Seoul, Korea). Separation was performed on a C_{18} column (Zorbax Eclipse XDB-C18, 4×6×250 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) with a gradient system. The mobile phase was a mixture of methanol and deionized water at a ratio of 70:30 (v/v). The flow rate was 0.8 mL/min for 30 min, and the UV/VIS detector was set at 280 nm. Capsaicin and dihydrocapsaicin were quantified by means of calibration curves obtained from commercial standards of capsaicinoids of the following concentrations: 10, 30, 50, and 100 ppm.

Preparation of OC nanoemulsions by ultrasonication Pre-mixtures were prepared with varying compositions of OC, surfactant, and co-surfactant based on the weight of each component. The samples that maintained a transparent one-phase after 4 weeks were considered to be nanoemulsions. Phase diagrams were constructed in the following manner: the pre-mixtures consisting of 3 components were prepared with OC, water, and Tween 80 or water+PG (2:1) or water+SM (100:3) at a constant weight ratio, respectively. The OC and Tween 80 were mixed at different ratios and stirred homogeneously for 3 min to give a uniform mixture. The pre-mixture containing OC, surfactant, and aqueous phase was heated at 50°C for 1 min and then sonicated at 46% amplitude for 3 min using an ultrasonicator (Sonic dismembrator, Model 500; Fisher Scientific, PA, USA). The samples were filtered and stored in a dark room and then diluted with distilled water (Fig. 1). After storing the emulsions for 28 days, the particle

Table 1. Effects of the mixing ratios of oleoresin capsicum (OC), ethyl alcohol, and Tweens on the formation of nanoemulsions

Mixing ratio				Appearance of nanoemusion formation				
Tween		EtOH	OC	Dispersed	Flocculated	Clump & separated	Opaque	Transparent
Tween 20	1	0	1	×	○	○	○	×
	2		1	×	○	○	○	×
	3		1	×	○	○	○	×
	1	1	1	×	○	○	○	×
	2		1	△	○	○	○	×
	3		1	△	○	○	○	×
	1	2	1	△	○	○	○	×
	2		1	△	○	○	○	×
	3		1	△	○	○	○	×
Tween 60	1	0	1	△	○	○	○	×
	2		1	△	△	△	○	×
	3		1	△	×	×	△	△
	1	1	1	○	○	○	○	×
	2		1	○	○	○	○	×
	3		1	○	○	○	○	×
	1	2	1	○	○	○	○	×
	2		1	○	○	○	○	×
	3		1	○	○	○	○	×
Tween 80	1	0	1	△	○	○	○	×
	2		1	△	△	△	○	×
	3		1	△	×	×	×	○
	1	1	1	○	○	○	○	×
	2		1	○	○	○	○	×
	3		1	○	○	○	○	×
	1	2	1	○	○	○	○	×
	2		1	○	○	○	○	×
	3		1	○	○	○	○	×

sizes of the nanoemulsions were determined. Finally, ternary-phase diagrams of the 3-component systems were constructed on a w/w basis to represent the points at which transparent, one-phase systems were found.

Screening of surfactants for nanoemulsions The OC, water, and surfactant were grouped in 3 different combinations for the phase diagrams (Table 1). Various hydrophilic surfactants and co-surfactants, such as Tween 20, Tween 40, Tween 60, Tween 80, SM, PG, and ethanol were tested to obtain a stable O/W nanoemulsion. Ternary mixtures with varying types and compositions of surfactant were pre-tested, and then a suitable surfactant and co-surfactant were selected for the preparation of the nanoemulsions.

Preparation of OC nanoemulsions by self-assembly The nanoemulsions were prepared by modified self-assembly emulsification according to the following briefly described procedure (27,28). The OC and Tween 80 were mixed at different weight ratios (1:1, 1:2, 1:3, 1:4, and 1:5) as indicated in Table 2. These mixture ratios were chosen to reflect increasing concentrations of surfactant with respect to OC for detailed study of the phase diagrams for nanoemulsion formation. The isotropic mixture (0.5 g) was accurately weighed and diluted with aqueous phase (100 mL) under moderate magnetic stirring for 2 hr, forming the nanoemulsion. Finally, the nanoemulsions were filtered and allowed to equilibrate for 24 hr before analysis (Fig. 1).

Determination of particle size distribution The particle size and size distribution of the nanoemulsions were determined by low-angle dynamic laser light scattering using a Nanotrac NPA 250 (Microtrac Inc., Montgomeryville, PA, USA).

Transmission electron microscopy (TEM) For negative staining, 7 μL of these annealed nanoemulsions were placed on carbon-coated copper grids and washed once with ultrapure water. Immediately the samples were stained with 7 μL of 2% uranyl acetate in ultrapure water, washed with water again, and then air dried. The grids were then examined by a transmission electron microscope (TECNAI 12; Phillips, Eindhoven, The Netherlands). Images were acquired at 120 kV and the scales were calibrated with a grating replica 3 mm grid BioScan camera (Model 792, GATAN; Bioscan Ltd.,Washington, DC, USA), and Digital

Table 2. Particle sizes of nanoemulsions prepared based on oleoresin capsicum (OC) and Tween 80 as the oil phase at different water contents by ultrasonication

Mixing ratio			Composition (%, w/w)			
OC	Tween 80	Water	OC	Tween 80	Water	Particle size (nm)[1)]
1	0.3	0.5	55.6	16.7	27.8	264±15.7
		1	43.5	13.0	43.5	234±58.2
		1.3	38.5	11.5	50.0	204±14.6
		1.5	35.7	10.7	53.6	193±23.1
		2	30.3	9.09	60.6	216±32.6
		3	23.3	6.98	69.8	252±8.57
1	0.5	1	40.0	20.0	40.0	155±9.42
		1.3	35.7	17.9	46.4	125±27.9
		1.5	33.3	16.7	50.0	152±16.0
		2	28.6	14.3	57.1	159±11.4
		3	22.2	11.1	66.7	156±32.2
1	0.7	1	37.0	25.9	37.0	192±11.3
		1.3	33.3	23.3	43.3	161±9.71
		1.5	31.3	21.9	46.9	140±20.3
		2	27.0	18.9	54.1	82.8±15.8
		3	21.3	14.9	63.8	34.8±1.03
		4	17.5	12.9	70.2	82.3±16.9
		5	14.9	10.5	74.6	77.5±4.30
		10	8.55	5.98	85.5	65.8±6.41
		50	1.93	1.35	96.7	69.1±0.55
1	1.0	1.3	30.3	30.3	39.4	239±3.54
		1.5	28.6	28.6	42.9	249±10.4
		2	25.0	25.0	50.0	162±4.65
		3	20.0	20.0	60.0	38.6±2.95
		4	16.7	16.7	66.7	25.7±1.44
		5	14.3	14.3	71.4	28.0±1.10
		10	8.33	8.33	83.3	30.7±1.91
		50	1.92	1.93	96.2	21.9±1.83
1	1.5	1	28.6	42.9	28.6	382±22.0
		1.3	26.3	39.5	34.2	364±76.7
		1.5	25.0	37.5	37.5	258±5.61
		2	22.2	33.3	44.4	164±2.84
		3	18.2	27.3	54.6	66.4±2.82
		4	15.3	23.1	61.5	15.5±0.48

1)All values are expressed as mean±SD of 5 replicate samples.

Micrograph software (Gatan v 3.4) was used for image capture.

Stability of nanoemulsions The stability of the nanoemulsions was evaluated by measuring the particle size by low angle dynamic laser light scattering using a Nanotrac NPA 250 (Microtrac Inc.), during storage at 25°C for 4 weeks.

Data analysis The results of the measurements (n=3) were analyzed statistically using SAS for Windows V8 (Statistical Analysis Systems, Cary, NC, USA). Analysis of variance and Duncan's multiple range test were employed to determine if the values were significantly different (p<0.05, p<0.01, and p<0.001).

Results and Discussion

Screening of surfactants for emulsion formation Nonionic surfactants are commonly used in the food industry, including monoglycerides, Tweens, and polysorbates. Of these, Tweens are widely used in food emulsion systems, primarily due to their relatively low toxicity, irritation potential, and the ease with which they form micro- or nanoemulsions. In order to identify the effect of a series of Tweens on emulsion formation, different Tweens were used as surfactants with mixtures composed of ethyl alcohol and OC. Table 1 shows the screening test as evaluated by macroscopy for the selection of a suitable Tween in the formation and stability of emulsions. The emulsions prepared with Tween 20 did not exhibit

Table 3. Particle sizes of nanoemulsions prepared based on oleoresin capsicum (OC) and Tween 80 as the oil phase at different ratios of water and propylene glycol (PG) by ultrasonication

Mixing ratio			Composition (%, w/w)				
OC	Tween 80	Water:PG (2:1)	OC	Tween 80	PG	Water	Particle size[1)] (nm)
1	0.3	0.5	55.6	16.7	9.26	18.5	331±63.7
		1	43.5	13.0	14.5	29.0	154±9.87
		1.3	38.5	11.5	16.7	33.3	148±11.1
		1.5	35.7	10.7	17.9	35.7	150±7.77
		2	30.3	9.09	20.2	40.4	136±14.7
		3	23.3	6.98	23.3	46.5	131±14.1
1	0.5	1	40.0	20.0	13.3	26.7	138±8.76
		1.3	35.7	17.9	15.5	31.0	157±3.66
		1.5	33.3	16.7	16.7	33.3	114±2.85
		2	28.6	14.3	19.1	38.1	77.7±4.40
		3	22.2	11.1	22.2	44.4	58.8±3.32
		4	18.2	9.09	24.2	48.5	89.9±6.54
		5	15.4	7.69	25.6	51.3	87.9±5.55
		10	8.70	4.35	29.0	58.0	102±9.20
		50	1.94	0.97	32.4	64.7	92.4±3.37
1	0.7	1	37.0	25.9	12.4	24.7	240±7.43
		1.3	33.3	23.3	14.4	28.9	88.1±8.77
		1.5	31.3	21.9	15.6	31.3	216±25.5
		2	27.0	18.9	18.0	36.0	66.2±8.31
		3	21.3	14.9	21.3	42.6	35.4±1.06
		4	17.5	12.9	24.0	46.8	35.5±2.97
		5	14.9	10.5	24.9	49.8	37.2±0.44
		10	8.56	5.98	28.5	57.0	53.2±6.16
		50	1.94	1.35	32.2	64.5	62.8±9.03
1	1.0	2	25.0	25.0	16.7	33.3	252±196
		3	20.0	20.0	20.0	40.0	58.2±4.46
		4	16.7	16.7	22.2	44.4	25.1±0.53
		5	14.3	14.3	23.8	47.6	16.4±0.74
1	1.5	3	18.2	27.3	18.2	36.4	240±22.2
		4	15.4	23.1	20.5	41.0	70.1±0.93
		5	13.3	20.0	22.2	44.4	63.4±1.41

[1)]All values are expressed as mean±SD of 5 replicate samples.

transparency and showed flocculated, clumped, and separated patterns; in particular, the emulsions could not disperse well without ethyl alcohol. Tween 60 produced an emulsion that exhibited a relatively good dispersity; however, it was also not transparent and accompanied with a flocculated, clumped, and separated pattern. One of the 4 different surfactants used, namely Tween 80, also produced emulsions with good dispersity at all ratios of each composition composed of ethyl alcohol and OC, with the exception of the transparency property. In particular, the emulsion maintained a transparent phase system without ethyl alcohol when prepared as Tween:ethyl alcohol:OC (3:0:1). Surfactants with greater hydrophilicity can wrap and stabilize particles in an O/W emulsion more efficiently. Also increasing the concentration of the surfactant decreases the particle size of the emulsion (11). These results indicated that Tween 80 can be used as a suitable surfactant to produce OC nanoemulsions that are stable dispersion one-phase systems, and the determined emulsification efficiencies clearly distinguished the ability of the various surfactants to emulsify OC. Therefore, Tween 80 was used in subsequent experiments to study the effects of co-surfactants and preparation methods, including assembly and ultrasonication, on the properties of OC nanoemulsions.

Nanoemulsion formation by ultrasonication The relationship between the phase behaviour of mixtures for a surfactant-oil phase and its composition can be expressed with the aid of a ternary-phase diagram (29). OC, Tween 80, SM, and PG were used as surfactant-oil phase mixtures in 3 groups (Table 2-4) to study the nanoemulsion areas using phase diagrams. The ternary phase diagrams were constructed separately for each group, so that the O/W nanoemulsion regions could be identified.

Table 4. Particle sizes of nanoemulsions prepared based on oleoresin capsicum (OC) and Tween 80 as the oil phase at different ratios of water and sucrose monostearate (SM) by ultrasonication

Mixing ratio			Composition (%, w/w)				
OC	Tween 80	Water:SM (100:3)	OC	Tween 80	SM	Water	Particle size[1)] (nm)
1	0.3	3	23.3	6.98	2.03	67.7	148±35.5
		4	18.9	5.66	2.20	73.3	155±40.5
		5	15.9	4.76	2.31	77.1	99.1±4.34
		10	8.85	2.65	2.50	85.9	42.4±1.99
		50	1.95	0.58	2.84	94.6	43.7±3.74
1	0.5	1.3	35.7	17.9	1.35	45.1	169±3.47
		1.5	33.3	16.7	1.46	48.5	88.4±11.9
		2	28.6	14.3	1.66	55.5	99.8±32.2
		3	22.2	11.1	1.94	64.7	125±39.4
		4	18.2	9.09	2.12	70.6	90.3±19.7
		5	15.4	7.69	2.24	74.7	59.4±18.1
		10	8.70	4.35	2.53	84.4	16.0±2.99
		50	1.94	0.97	2.83	94.3	4.73±0.27
1	0.7	1.5	31.3	21.9	1.37	45.5	135±9.96
		2	27.0	18.9	1.57	52.5	66.2±8.74
		3	21.3	14.9	1.86	62.0	35.0±6.79
		4	17.5	12.9	2.04	68.1	31.9±3.61
		5	14.9	10.5	2.17	72.5	25.2±1.21
		10	8.55	5.98	2.49	83.0	17.7±0.04
		50	1.93	1.35	2.82	93.9	6.80±0.17
1	1	2	25.0	25.0	1.46	48.5	123±9.49
		3	20.0	20.0	1.75	58.3	34.6±0.95
		4	16.7	16.7	1.94	64.7	27.1±1.63
1	1.5	3	18.2	27.3	1.59	53.0	53.8±1.45
		4	15.4	23.1	1.79	59.8	33.7±0.55

[1)]All values are expressed as mean±SD of 5 replicate samples.

Nanoemulsion formation on the system OC/Tween 80/water Table 2 shows the particle size distributions of the nanoemulsions prepared from the system OC/Tween 80/water, as a function of the Tween 80 concentration at different weight ratios of Tween 80C/water and at a constant OC weight ratio. It can be seen that the OC:Tween 80 ratios of 1:0.3 and 1:0.5 resulted in nanoemulsions of particle sizes ranging between 125 and 264 nm. However, as the OC:Tween 80 ratio increased from a ratio of 1:0.7 to 1:1, and to a ratio of 1:1.5, the particle sizes of the nanoemulsions were mostly smaller than 100 nm. These results indicated that the concentration of Tween 80 might greatly affect particle size distribution in the formation of nanoemulsions. Therefore, in preparing nanoemulsions from the system of OC/Tween 80/water, the particle sizes were smaller than 100 nm in the aqueous phase at 1.92-27.0% OC, 1.93-27.3% Tween 80, and 54.1-96.2% water. The ratio of 1:0.7:2 for the system OC/Tween 80/water (27.0%:18.9%:54.1%), containing the maximum amount of OC, formed a nanoemulsion of maximum particle size (82.8±15.8 nm), whereas the ratio of 1:1.5:4 (15.3%:23.1%:61.5%) formed a nanoemulsion of minimum particle size (15.5±0.48 nm). It was clear that increasing the concentration of surfactant versus OC lead to decreasing particle size, indicating that the particle size distribution was inversely proportional to the concentration of surfactant. This might be due to the fact that smaller particle sizes imply greater surface areas, which would require more surfactant to cover (10). The phase diagram of the 3-component system OC/Tween 80/water is presented in Fig. 2. The phase boundary was determined by characterizing the particle size of the nanoemulsion. The white area is the one-phase, the transparent region of the nanoemulsion ranging below 100 nm. The grey area is the multiple, cloudy region of the emulsion, ranging between 100 and 400 nm. Finally, the dark grey area is the region of phase separation, which occurred in both the OC and Tween 80 corners of the phase diagram. These results clearly demonstrate that a nanoemulsion can be formed from the system OC/Tween 80/water without the addition of a co-surfactant or solvent, presumably due to the presence of fatty acids in the OC, which tend to act as solvents. Therefore, in terms of both the cost and safety of emulsion ingredients, this system may be beneficial in formulating nanoemulsions for food use due to its simple composition and low concentration.

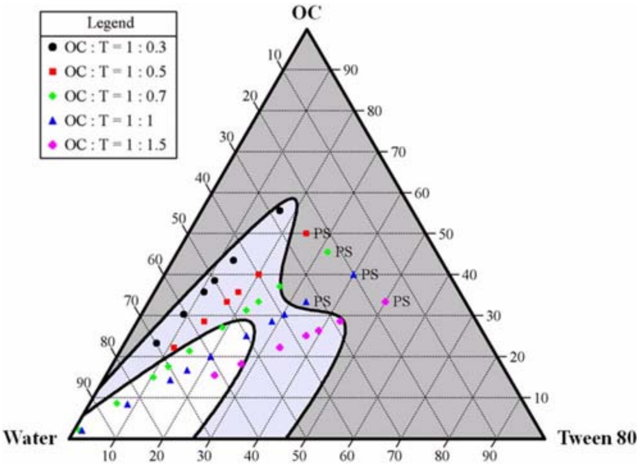

Fig. 2. Ternary-phase diagram of nanoemulsions formed by ultrasonication with the system OC/Tween 80/water. OC, oleoresin capsicum; T, Tween 80; PS, phase separated.

Effect of partial substitution of PG on the system OC/ Tween 80/water+PG In order to improve the functional properties of emulsions in the food industry, surfactants are often used in combinations with other types of surfactants, short-chain alcohols (such as ethanol), and polyols (glycerol or PG), rather than as individual components. Tweens are food grade surfactants with a negative image. In many countries there is constant pressure to remove them from key food formulations. Of all the polyols, PG is similar to water, because it forms hydrogen bonds and has a relatively high dielectric constant. However, PG is unfavorable in large quantities for food purposes (the limit in food is 1 g/kg) (30). Therefore, there is a need to find ways to reduce the use of PG and Tween 80 while also producing a clear, one-phase nanoemulsion region. The formulations for the system of OC/Tween 80/water+PG (2:1) were prepared with Tween 80 and PG as the surfactant and solvent, respectively (Table 3). It can be seen that the OC:Tween 80 ratios of 1:0.5, 1:0.7, 1:1, and 1:1.5 resulted in nanoemulsions of particle sizes ranging below 100 nm. In the system of OC/Tween 80/water+PG there were small increases in the nanoemulsion region as compared to the system of OC/ Tween 80/water co-surfactant. The concentration of the aqueous phase required a minimum 2-3 fold amount of surfactant, which seemed to be similar to the system of OC/Tween 80/water. The OC:Tween 80:water+PG ratio of 1:0.7:1.3 (33.3%:23.3%:43.3%), containing a maximum amount of OC, formed a nanoemulsion with a maximum particle size of 88.1±8.77 nm, whereas the ratio of 1:1:5 (14.3%:14.3%:71.4%) formed a nanoemulsion with a minimum particle size of 16.4±0.74 nm. In this system, the amount of OC in the nanoemulsion was maximally reached at 33.3% as compared to 27.0% in the system of OC/Tween 80/water. Consequently, OC nanoemulsions were formed with 1.94-33.3% OC, 0.97-23.3% Tween 80, and 43.3-97.1% water+PG in the system. The phase diagram changes of the OC/Tween 80/water+PG systems are shown in Fig. 3. The white, grey, and dark grey areas exhibit the one-phase nanoemulsion (below 100 nm) region; the multiple, cloudy region (between 100 and 400 nm); and the region of phase separation, respectively.

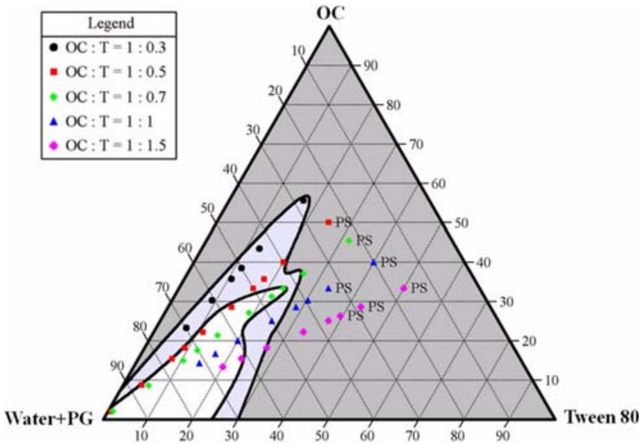

Fig. 3. Ternary-phase diagram of nanoemulsions formed by ultrasonication with the system OC/Tween 80/water+PG. OC, oleoresin capsicum; T, Tween 80; PG, propylene glycol; PS, phase separated.

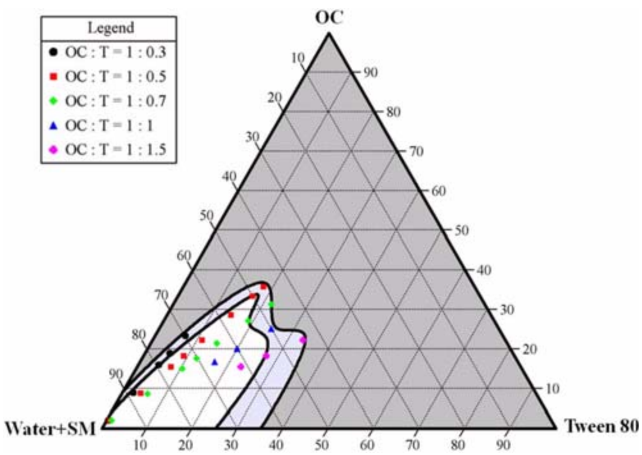

Fig. 4. Ternary-phase diagram of nanoemulsions formed by ultrasonication with the system OC/Tween 80/water+SM. OC, oleoresin capsicum; T, Tween 80; SM, sucrose monostearate.

The extent of nanoemulsion existence and the separation region were similar to those observed using the system OC/Tween 80/water. By the addition of PG to replace the water in the system, there was a large difference in the extents of the one- and multiple-phases, which showed that the OC/Tween 80/water+PG system exhibited a slightly smaller region than the system OC/Tween 80/water. In addition, both the one and multiple-phases were formed when the amount of Tween 80 was below 30%, which showed the effect of replacing the Tween 80. These results correlated with the research of Patel *et al.* (30) who found that when replacing PG with water, even as little as 10 wt % water significantly reduced the extent of the clear, one-phase nanoemulsion region and increased the liquid crystal region in emulsion systems composed of lecithin, medium chain triglyceride, and PG+water.

Effect of partial substitution of SM on the system OC/ Tween 80/water+SM To study the effects of SM replacement on OC nanoemulsion formation, we prepared

Table 5. Particle sizes of nanoemulsions prepared based on oleoresin capsicum (OC) and Tween 80 as the oil phase at different water contents by self-assembly

Mixing ratio			Composition (%, w/w)			
OC	Tween 80	Water	OC	Tween 80	Water	Particle size[1)] (nm)
1	2	100	0.17	0.33	99.5	853±682
		50	0.33	0.66	99.0	330±125
		20	0.81	1.63	97.6	1550±253
		10	1.59	3.17	95.2	1161±616
		5	3.03	6.06	90.9	2-phase
		3	4.76	9.52	85.7	2-phase
1	3	100	0.12	0.37	99.5	17.4±0.35
		50	0.25	0.74	99.0	16.6±0.15
		20	0.61	1.83	97.6	15.2±1.19
		10	1.19	3.57	95.2	16.3±2.05
		5	2.27	6.82	90.9	95.7±80.7
		3	3.57	10.7	85.7	1395±125
1	4	100	0.10	0.40	99.5	16.6±0.25
		50	0.20	0.79	99.0	15.8±0.03
		20	0.49	1.95	97.6	14.5±0.15
		10	0.95	3.81	95.2	13.6±0.07
		5	1.82	7.27	90.9	13.6±0.21
		3	2.86	11.43	85.7	11.5±2.63
1	5	100	0.08	0.41	99.5	15.4±0.02
		50	0.17	0.83	99.0	14.8±0.16
		20	0.41	2.03	97.6	13.6±0.10
		10	0.79	3.97	95.2	13.1±0.07
		5	1.52	7.58	90.9	13.1±0.06
		3	2.38	11.90	85.7	12.6±0.07

[1)]All values are expressed as mean±SD of 5 replicate samples.

a homogenous aqueous phase with a mixture of OC, Tween 80, and water+SM (100:3). It can be seen that the OC:Tween 80 ratios of 1:0.3, 1:0.5, 1:0.7, 1:1, and 1:1.5 resulted in nanoemulsions of particle sizes ranging below 100 nm (Table 4). In the OC/Tween 80/water+SM system, there were small increases in the nanoemulsion region as compared to the OC/Tween 80/water system and OC/Tween 80/water+PG system (Fig. 4). The content of SM in the aqueous phase was extremely small, at 1.46-2.84%, as compared to that of PG in the OC/Tween 80/water+PG system, and SM was more effective in nanoemulsion formation. The OC, Tween 80, and water+SM ratio of 1:0.5:1.5 (33.3%:16.7%:50.0%), containing a maximum amount of OC, formed a nanoemulsion with a maximum particle size of 88.4±11.9 nm. SM as a co-surfactant was more effective than PG for the formation of an O/W nanoemulsion containing OC. This result may be due to the fact that PG is one of the least hydrophilic simple polyols, which are soluble in water but practically insoluble in the oil phase (31). Meanwhile, SM is more hydrophilic for dissolution into the oil phase and does not lose solubility during emulsion formation (32). The contents of Tween 80 and SM in the OC/Tween 80/water+SM system were not different from that of Tween 80 in the OC/Tween 80/water system without a co-surfactant. Interestingly, however, it has not been firmly established whether the same sequence of phase behavior could be expected upon the replacement of water with an alternative polar solvent. These results are in good agreement with those reported by Jafari *et al.* (33).

Nanoemulsion formation by self-assembly The nanoemulsion by self-assembly was prepared using Tween 80 alone, which was used to select the optimum conditions for an O/W nanoemulsion containing OC prepared by ultrasonication. The nanoemulsion region formed below 100 nm in the nanoemulsion prepared by self-assembly, and it was more reduced than the nanoemulsion prepared by ultrasonication. Nanoemulsions were obtained with the OC/Tween 80/water system at ranges of 0.08-2.86, 0.37-11.90, and 85.7-99.5%, respectively (Table 5). The nanoemulsion region was formed under the condition of more than 85.7% water content (Fig. 5). The OC/Tween 80/water ratio of 1:3:10 (1.19%:3.57%:95.2%), containing a minimum amount of surfactant, formed a nanoemulsion with a particle size of 16.3±2.05 nm. However, by decreasing the ratio of the OC/Tween 80/water mixture (1:2:10), a nanoemulsion was formed with a size range of over 300 nm and the oil phase was separated from the aqueous phase. The presence of co-surfactants decreases the bending stress of the interface and allows the interfacial film sufficient flexibility to take up different curvatures required

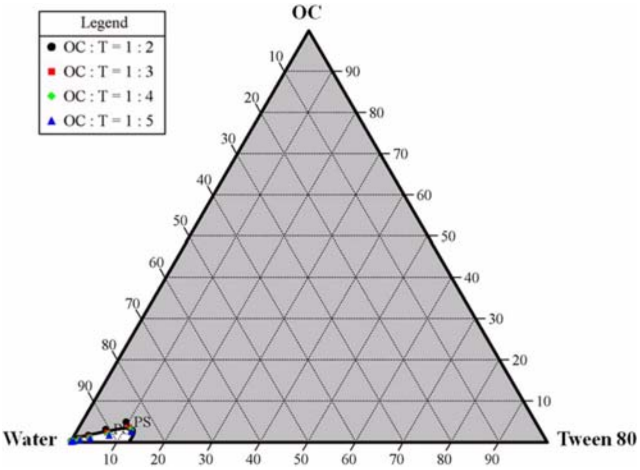

Fig. 5. Ternary-phase diagram of nanoemulsions formed by self-assembly with the system OC/Tween 80/water. OC, oleoresin capsicum; T, Tween 80; PS, phase separated.

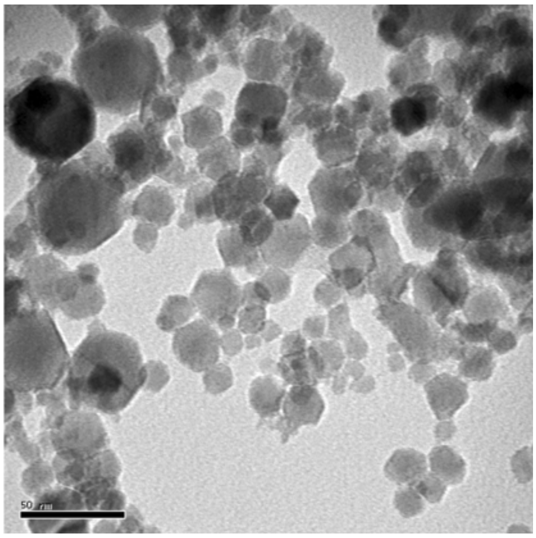

Fig. 6. TEM of a nanoemulsion prepared by self-assembly with an OC/Tween 80/water ratio of 1:1.5:4.

to form nanoemulsions over a wide range of compositions (34). However, in the present study, the OC nanoemulsion prepared with co-surfactant was not different from that prepared with Tween 80 only. Thus, co-surfactants were not used to prepare the nanoemulsion by self-assembly. According to these results, it is suggested that ultrasonication was more effective either by increasing the content of OC or by decreasing the content of surfactant than self-assembly.

TEM of nanoemulsion prepared by self-assembly Morphological observation of the nanoemulsion prepared by self-assembly was conducted using TEM (Fig. 6). The TEM image indicated the presence of a droplet-type nanoemulsion structure, which exists as a spherical nanoemulsion. Most nanoemulsion droplets are confirmed to have uniform sizes of below 50 nm, which in correspondence, if the packing parameter in an emulsion is smaller than 1, normal micelles are formed near 50 nm in O/W self-assembly structures. The packing parameter is defined as the shape of molecules with the dimensionless packing parameter P=n/*al,* n is the effective (or hydrated) cross-sectional area of the polar head group. Therefore, the packing parameter (the self-assembly structure) can change with parameters such as temperature and solvent conditions (35).

Stability of nanoemulsions during storage The size changes of nanoemulsions preprared by ultrasonication in the OC/Tween 80/water system over a 28 day storage period at 25°C are shown in Table 6. Most of the nanoemulsions prepared from the OC/Tween 80/water

Table 6. Particle sizes of nanoemulsions prepared by ultrasonication depending on the combination of OC/Tween 80/water during storage for 28 days

Mixture ratio	Storage (day)					F-value
	0	1	7	14	28	
1:0.7:2	82.8±15.8	96.2±5.23	80.5±19.39	91.7±3.80	85.4±4.50	0.92
1:0.7:3	34.8±1.03$^{a1)}$	29.2±0.62^{b}	14.38±0.96^{e}	24.1±0.61^{c}	19.0±1.30^{d}	223.14***
1:0.7:4	82.3±16.9	85.1±5.86	84.2±5.05	86.4±4.44	86.1±0.32	0.11
1:0.7:5	77.5±4.30	84.0±2.65	81.3±1.28	72.7±2.71	74.3±13.7	1.51
1:0.7:10	65.8±6.41	67.7±0.57	70.6±1.56	65.1±5.31	65.3±4.52	0.88
1:0.7:50	69.1±0.55^{c}	70.2±0.51^{bc}	71.2±1.85^{ab}	72.4±1.00^{a}	71.5±0.21^{d}	2410.48
1:1:3	38.6±2.95^{a}	37.3±2.05^{ab}	29.7±5.04^{c}	31.6±1.55^{bc}	40.7±4.40^{a}	5.57**
1:1:4	25.7±1.44^{c}	26.3±0.83^{c}	33.4±0.64^{a}	27.7±0.80^{bc}	30.7±3.38^{ab}	10.25**
1:1:5	28.0±1.10^{b}	29.4±2.43^{b}	34.4±0.21^{a}	29.5±1.00^{b}	31.2±2.87^{ab}	5.18*
1:1:10	30.7±1.91^{c}	18.4±0.53^{d}	37.3±1.61^{b}	41.7±2.07^{a}	30.0±2.16^{c}	75.99***
1:1:50	21.9±1.83^{e}	7.84±0.37^{d}	31.9±1.71^{c}	47.6±1.21^{a}	43.2±0.06^{b}	497.82***
1:1.5:3	66.4±2.82^{c}	69.2±0.06^{c}	66.5±2.81^{c}	174±4.04^{b}	257±2.08^{a}	3067.65***
1:1.5:4	15.5±0.48^{e}	13.9±0.30^{d}	20.4±0.20^{c}	28.3±0.84^{a}	23.6±0.68^{b}	341.71***

[1)]Means designated with different letters are significantly different from each other within a row at α=0.05 as determined by Duncan's multiple range test (*p<0.05; **p<0.01; ***p<0.001).

Table 7. Particle sizes of nanoemulsions prepared by ultrasonication depending on the combination of OC/Tween 80/water+PG during storage for 28 days

Mixture ratio	Storage (day)					F-value
	0	1	7	14	28	
1:0.5:2	77.7±4.40$^{c1)}$	77.7±1.16^{c}	75.4±1.55^{c}	87.8±2.12^{b}	98.2±7.10^{a}	17.80***
1:0.5:3	58.8±3.32	65.0±10.1	62.7±7.49	66.9±2.06	65.4±6.40	0.70
1:0.5:4	89.9±6.54	77.7±17.5	74.6±6.60	75.6±10.6	89.8±16.2	1.20
1:0.5:5	87.9±5.55	77.4±9.87	82.7±18.4	87.9±2.16	76.5±8.21	0.84
1:0.5:10	102±9.20^{ab}	117±7.26^{a}	109±9.65^{a}	100±8.14^{ab}	88.0±9.34^{b}	4.50*
1:0.5:50	92.4±3.37	95.9±16.1	104±5.67	89.7±25.5	108±10.9	0.81
1:0.7:2	66.2±8.31^{b}	85.4±4.20^{a}	84.0±2.26^{a}	83.3±2.75^{a}	81.4±1.80^{a}	9.07*
1:0.7:3	35.5±1.06	37.0±2.00	40.7±2.54	42.1±2.11	63.3±33.2	1.72
1:0.7:4	35.4±2.97^{b}	35.7±2.75^{b}	41.1±3.03^{b}	43.2±2.86^{b}	66.5±20.8^{a}	5.24*
1:0.7:5	37.2±0.44^{c}	38.3±0.72^{bc}	44.8±5.84^{ab}	46.7±1.21^{a}	43.1±4.56^{abc}	4.47*
1:0.7:10	53.2±6.16^{b}	54.8±7.62^{ab}	65.1±2.87^{a}	65.1±4.92^{a}	62.7±4.05^{ab}	3.42
1:0.7:50	62.8±9.03^{b}	36.3±10.7^{c}	77.2±2.81^{a}	76.8±2.64^{a}	86.9±7.79^{a}	21.45***
1:1:3	58.2±4.46^{a}	56.4±0.31^{a}	55.8±1.74^{a}	56.8±2.80^{a}	43.1±4.56^{b}	11.00**
1:1:4	25.1±0.53^{c}	29.1±0.58^{b}	32.3±0.86^{b}	31.4±1.25^{b}	62.7±4.05^{a}	177.24***
1:1:5	16.4±0.74^{d}	27.5±2.56^{c}	28.8±1.24^{c}	31.9±0.95^{b}	36.3±1.07^{a}	76.85***
1:1.5:4	70.1±0.93^{d}	82.5±2.60^{d}	108±0.98^{c}	142±4.12^{b}	198±9.37^{a}	385.90***
1:1.5:5	63.4±1.41^{d}	61.0±0.95^{d}	90.0±3.46^{c}	93.8±0.17^{b}	119±2.19^{a}	437.38***

[1)]Means designated with different letters are significantly different from each other within a row at α=0.05 as determined by Duncan's multiple range test (*p<0.05; **p<0.01; ***p<0.001).

Table 8. Particle sizes of nanoemulsions prepared by ultrasonication depending on the combination of OC/Tween 80/water+SM during storage for 28 days

Mixture ratio	Storage (day)					F-value
	0	1	7	14	28	
1:0.3:5	99.1±4.34	96.0±0.67	107±23.0	108±6.51	147±69.3	1.19
1:0.3:10	42.4±1.99$^{b1)}$	44.0±0.38^{b}	36.4±1.01^{d}	72.7±0.90^{a}	39.6±1.08^{c}	454.39***
1:0.3:50	43.7±3.74^{b}	74.5±17.5^{a}	45.2±22.2^{b}	8.96±0.20^{c}	14.0±0.77^{c}	13.12***
1:0.5:1.5	88.4±11.9	132±24.1	132±51.6	128±12.6	100±4.43	1.74
1:0.5:2	99.8±32.2	95.6±1.35	99.7±36.9	94.7±1.35	90.8±21.7	0.07
1:0.5:4	90.3±19.7	93.8±16.2	99.7±18.8	96.3±2.40	89.0±2.76	0.28
1:0.5:5	59.4±18.1	76.7±13.8	66.6±13.1	65.5±25.0	55.6±6.93	0.73
1:0.5:10	16.0±2.99^{c}	35.2±4.73^{a}	31.9±0.90^{a}	30.3±2.00^{a}	22.5±4.84^{b}	15.36***
1:0.5:50	4.68±0.00^{c}	12.6±1.55^{b}	13.3±2.44^{b}	19.6±5.99^{a}	5.21±0.10^{c}	13.18***
1:0.7:2	66.2±8.74	104±41.0	66.4±2.46	71±11.51	65.2±6.55	2.11
1:0.7:3	35.0±6.79^{b}	55.9±23.2^{ab}	48.1±8.01^{ab}	64.6±6.12^{a}	57.8±2.93^{ab}	2.76
1:0.7:4	31.9±3.61^{b}	54.4±10.4^{a}	56.6±1.37^{a}	51.0±18.1^{ab}	50.8±2.56^{ab}	3.19
1:0.7:5	25.2±1.21^{b}	45.4±14.5^{ab}	46.2±24.3^{ab}	54.5±3.06^{a}	36.1±2.16^{ab}	2.30
1:0.7:10	17.7±0.04^{d}	29.8±1.07^{a}	26.3±1.80^{bc}	27.4±1.73^{ab}	24.2±1.71^{c}	30.45***
1:0.7:50	6.84±0.17^{c}	11.4±2.21^{ab}	8.07±1.82^{c}	12.5±1.67^{a}	8.93±0.22^{bc}	7.56**
1:1:3	34.6±0.95^{b}	35.3±2.20^{b}	21.4±1.93^{d}	26.3±1.91^{c}	40.4±3.29^{a}	37.45***
1:1:4	27.1±1.63^{a}	25.2±0.96^{a}	12.61±0.23^{b}	8.11±6.81^{b}	24.2±0.38^{a}	21.61***
1:1.5:3	53.8±1.45^{c}	51.1±3.78^{c}	47.0±2.43^{c}	197±80.9^{b}	354±39.6^{a}	33.51***
1:1.5:4	35.8±0.55^{ab}	52.5±2.97^{ab}	14.3±2.37^{b}	75.8±61.7^{a}	26.2±3.47^{ab}	2.25

[1)]Means designated with different letters are significantly different from each other within a row at α=0.05 as determined by Duncan's multiple range test (*p<0.05; **p<0.01; ***p<0.001).

system maintained their initial sizes without great change, ranging from 65 to 92 nm. Particularly, as the water weight ratio increased in the OC/Tween 80/water system, such as 1:1:3, 1:1:4, 1:1:5, 1:1:10, and 1:1:50, all the nanoemulsions maintained their sizes in the range of 30 to 48 nm. Generally, the ratio of Tween 80 affected nanoemulsion

Table 9. Particle sizes of nanoemulsions prepared by self-assembly depending on the combination of OC/Tween 80/water during storage for 28 days

Mixture ratio	Storage (day)								F-value
	0	1	3	5	7	14	21	28	
1:5:3	12.6±0.07$^{b1)}$	11.9±0.05^{d}	13.0±0.04^{a}	11.3±0.04^{f}	12.7±0.02^{b}	12.3±0.35^{c}	11.4±0.04^{ef}	11.6±0.16^{e}	40.69***
1:5:5	13.1±0.06^{c}	12.5±0.08^{de}	13.4±0.13^{bc}	12.3±0.17^{e}	13.9±0.04^{a}	13.4±0.22^{bc}	12.6±0.39^{d}	13.7±0.12^{ab}	20.39***
1:5:10	13.1±0.07^{e}	12.6±0.06^{g}	13.5±0.10^{c}	12.1±0.03^{h}	13.4±0.07^{d}	12.8±0.03^{f}	13.8±0.11^{b}	13.9±0.05^{a}	179.19***
1:5:20	13.6±0.10^{c}	12.8±0.13^{d}	14.1±0.04^{b}	12.7±0.02^{e}	14.6±0.03^{a}	13.7±0.08^{c}	13.6±0.09^{c}	12.2±0.01^{f}	300.64***
1:5:50	14.8±0.16^{c}	13.4±0.10^{e}	16.0±0.07^{b}	13.2±0.11^{f}	16.3±0.08^{a}	14.3±0.09^{d}	14.5±0.01^{d}	11.2±0.26^{g}	359.28***
1:5:100	15.4±0.02^{bc}	15.5±0.64^{bc}	16.1±0.48^{b}	11.3±0.49^{e}	17.7±0.08^{a}	14.1±0.31^{d}	15.0±0.16^{cd}	6.19±1.31^{f}	80.90***
1:4:3	11.5±2.63^{c}	13.9±0.02^{a}	13.2±0.03^{ab}	11.6±0.07^{bc}	13.8±0.03^{a}	11.9±0.04^{bc}	11.9±0.03^{bc}	11.1±0.13^{c}	4.20**
1:4:5	13.6±0.21^{a}	13.7±0.13^{a}	14.1±0.02^{a}	12.3±0.11^{ab}	14.2±0.05^{a}	13.3±0.17^{ab}	13.3±3.47^{b}	13.0±0.10^{ab}	2.12
1:4:10	13.6±0.07^{d}	13.6±0.07^{d}	14.3±0.09^{b}	12.1±0.13^{f}	14.3±0.09^{b}	13.3±0.07^{e}	13.3±0.15^{c}	16.1±0.02^{a}	308.03***
1:4:20	14.5±0.15^{b}	13.6±0.06^{d}	14.7±0.10^{b}	12.3±0.03^{f}	15.2±0.01^{a}	14.0±0.07^{c}	14.0±0.02^{cd}	13.1±0.36^{e}	84.48***
1:4:50	15.8±0.03^{c}	13.8±0.26^{f}	17.3±0.00^{b}	11.9±0.05^{h}	18.0±0.01^{a}	15.2±0.11^{d}	15.2±0.38^{e}	12.3±0.17^{g}	326.97***
1:4:100	16.6±0.25^{b}	12.1±0.09^{d}	18.9±0.09^{a}	9.61±0.25^{f}	18.5±0.22^{a}	15.6±0.13^{c}	15.6±0.11^{c}	10.2±0.65^{e}	334.30***
1:3:3	1395±125.3	1334±151.0	1317±14.73	1333±222.29	1713±237.0	1560±178.5	1301±141.2	1352±102.2	1.81
1:3:5	95.7±8.69^{a}	27.9±5.64^{b}	22.6±3.75^{b}	20.7±4.69^{b}	26.9±2.15^{b}	23.1±2.21^{b}	22.5±4.80^{b}	18.0±2.24^{b}	2.41
1:3:10	16.3±2.05	15.2±0.42	14.2±4.06	13.4±0.20	15.1±0.45	19.2±5.92	15.7±1.47	13.6±0.38	1.09
1:3:20	15.2±1.19^{c}	11.6±7.82^{c}	14.9±0.68^{c}	14.6±1.18^{c}	15.3±3.26^{c}	10.4±4.71^{c}	26.5±4.36^{b}	36.8±3.32^{a}	14.90***
1:3:50	16.6±0.15^{bc}	17.1±0.70^{b}	11.8±5.40^{cd}	13.9±0.69^{bc}	17.4±1.79^{b}	42.3±1.11^{a}	42.4±3.86^{a}	6.56±4.09^{d}	49.89***
1:3:100	17.4±0.35^{d}	45.1±3.16^{b}	35.7±3.41^{c}	14.4±4.20^{d}	14.4±4.20^{d}	22.1±9.10^{d}	36.8±3.84^{c}	59.3±2.85^{a}	32.98***

1) Means designated with different letters are significantly different from each other within a row at •·=0.05 as determined by Duncan's multiple range test (*p<0.05; **p<0.01; ***p<0.001).

stability significantly at the OC:Tween 80 ratios of 1:1 and 1:1.5, depending on the water content in the OC/Tween 80/water system. In the OC/Tween 80/water+PG system, the nanoemulsions with weight ratios of 1:0.5:2, 1:0.5:3, 1:0.5:4, 1:0.5:5, 1:0.5:10, and 1:0.5:50 maintained their particle sizes without the occurrence of flocculation (Table 7). Due to the presence of PG, the nanoemulsions exhibited a stable form not only at 1:1 but also at the 1:0.7 ratio of Tween 80:water in the OC/Tween 80/water+PG system. The storage stabilities of the OC/Tween 80/water+SM nanoemulsions are indicated in Table 8. The OC/Tween 80/water+SM nanoemulsion systema at OC/Tween 80 weight ratios of 1:0.3, 1:0.5, and 1:0.7 maintained their stability, and also the mixture of water+SM greatly affected the size of the nanoemulsions at ratios such as 1:0.3:5, 1:0.5:1.5, 1:0.5:10, 1:0.5:50, 1:0.7:10, and 1:0.7:50 in the OC/Tween 80/water+SM system.

The stabilities of the nanoemulsions formed in the OC/Tween 80/water system by self-assembly are presented in Table 9. The nanoemulsions formulated with OC and Tween 80 at ratios of 1:4 and 1:5, which varied in water ratio from 3 to 100, showed good stability during storage with sizes ranging from 11 to 19 nm. The addition of $CaCl_2$ into an emulsion can prevent depletion flocculation due to the electrostatic screening and bridging effects of calcium ions in O/W emulsions (36).

In conclusion, the preparation of O/W nanoemulsions containing OC, several kinds of surfactants such as Tween 80, PG, and SM could be used to solubilize OC and produce one or two-phase nanoemulsions with separation regions that were dependent on the formulation. Ultrasonication seems to be more powerful in forming nanoemulsions than self-assembly, which might be due to the formation capacity of the nanoemulsion phase. However, we also suggest that self-assembly is a useful method to prepare O/W nanoemulsions containing OC due to the low production cost and stability of such nanoemulsions. The optimal conditions for the preparation of food nanoemulsions by ultrasonication were confirmed as a mixture of OC:Tween 80:water=1:0.7:2 (27.0%:18.9%:54.1%), and the diameter of the droplets was 82.8±15.8 nm. A nanoemulsion was formed by self-assembly using a mixture ratio of OC:Tween 80:water=1:3:5 (1.19%:3.57%:95.2%), and the diameter of the droplets was 16.3±2.05 nm. In conclusion, OC nanoemulsions prepared with Tween 80 only, by self-assembly, can be expected to have improved formation ability and storage stability, and therefore, may be applicable in the production of functional foods containing nutraceutical ingredients. In addition, they may provide novel active lipophilic functionalities such as solubility, bioavailability, biocompatibility, and stability in a bioactives delivery system.

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