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Determination of Required HLB Values for *Citrus unshiu* Fruit Oil, *Citrus unshiu* Peel Oil, Horse Fat and *Camellia japonica* Seed Oil

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Synopsis

In the present study, the required hydrophilic lipophilic balance (HLB) values of *Citrus unshiu* fruit oil (CUFO), *Citrus unshiu* peel oil (CUPO), horse fat (HF), and *Camellia japonica* seed oil were determined empirically by preparing oil-in-water (O/W) emulsions. Lipophilic and hydrophilic surfactants were prepared in various ratios in O/W emulsion. The droplet size of the emulsion was measured using a particle size analyzer, and the turbidity was measured using a turbidity meter and a ultraviolet (UV)-vis spectrophotometer. According to the Orafidiya–Oladimeji method, the HLB value of the emulsion having the minimum dispersion ratio, the minimum droplet size, and the maximum turbidity degree was determined as the required HLB value for each essential oil. Based on these methods, the required HLB values of CUFO, CUPO, HF, and *Camellia japonica* seed oil were determined as 14.75–14.90, 15.35–15.40, 6.30–7.06, and 5.94–6.30, respectively.

INTRODUCTION

Essential oils are commonly used as preservatives and fragrances in cosmetics. However, in recent years, the medical values of essential oils such as antimicrobial properties, and their other various applications, for example, as food additives, organic pesticides, and natural insecticides are being actively explored. The oil-in-water (O/W) emulsion is one of the preferred techniques to minimize evaporation of the volatile essential oils, and thereby increase their shelf life (1–4). However, the O/W emulsion is thermodynamically unstable and can lead to various types of phase separations. These phase separation phenomenon can pose a problem to the functionality and stability of the active ingredient in the product, so the emulsification technology in cosmetics is very important (5–7). To produce a stable emulsion, emulsifiers or combinations of emulsifiers are used with the required hydrophilic lipophilic balance (HLB) values depending on the oil phase. Optimal stability is achieved when the HLB value of the surfactant mixture is close to that of the required oil (8–13). Required HLB means that when two or more emulsifiers

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are mixed with a given oil, the HLB value is presented as the optimum ratio that provides optimum emulsification (14–18). $\mathrm{HLB}_{\mathrm{required}} = \sum \mathrm{HLB}_{\mathrm{required}(i)} \times f_i$, where f_i is the mass (or weight) fraction of the oil i. The required HLB values are used not only to calculate the required HLB values of the oil phase but also to estimate the values of each component of the oil phase. HLB values are known to be affected by significant errors. Therefore, the required HLB values should be determined experimentally (19,20).

Experiments were conducted using three types of essential oils from Jeju Island and Jeju horse fat (HF). *Citrus unshiu* fruit oil (CUFO) has antibacterial activity against the main causes of acne (21). *Citrus unshiu* peel oil (CUPO) has physiological activities, such as antioxidant and antibacterial activities (22). *Camellia japonica* seed oil (CJSO) has a wrinkle improvement effect by inhibiting matrix metalloproteinase activity and by inducing human type I procollagen synthesis (23). In addition, *Camellia japonica* seed oil is known to have a dietary effect by reducing low-density lipoprotein cholesterol level and increasing high-density lipoprotein cholesterol level (24). HF could be used as cosmetic and pharmaceutical materials with anti-inflammatory effects by reducing of erythema and Immunoglobulin E level and recovering of histopathological features of skin (25).

In the present study, to determine the required HLB value of essential oils, the method of Orafidiya and Oladimeji was used in which stable emulsions were based on minimum droplet size and maximum turbidity (1). To verify our results, we also carried out related experiments using liquid paraffin as a reference standard.

MATERIALS AND METHODS

MATERIALS

Liquid paraffin (paraffin oil, Sigma-Aldrich, St. Louis, MO), sorbitan sesquioleate (Rheodol AO-15V, Kao, Tokyo, Japan; HLB = 3.70), polysorbate 60 (Rheodol TW-S120V, Kao, Tokyo, Japan; HLB = 14.90), and polysorbate 20 (Rheodol TW-L120V, Kao; HLB = 16.70) were used. *Citrus unshiu* fruit oil (Human Island, Jeju, Korea), *Citrus unshiu* peel oil (Human Island), horse fat (Ecotech, Bucheon, Korea), *Camellia japonica* seed oil (CJSO, Daebongs, Incheon, Korea), and pure water ($k < 3$ μS/cm, Pure RO 130, Human Corporation) were used. See Tables 1 and 2.

Table I
Description of Raw Materials Used

INCI name	Science name	Manufacturing method	Specific gravity (d20/20)	Refraction index[a]
Citrus unshiu fruit oil	*Citrus unshiu* Markovich	Steam distillation	0.856	1.471
Citrus unshiu peel oil	*Citrus unshiu* Markovich	Cold press	0.862	1.470
Horse fat	Horse fat	Fermentation	0.860	1.469
Camellia japonica seed oil	*Camellia japonica* L.	Cold press	0.911	1.467

[a]Refractometer (NAR-1T, Atago, Japan). INCI, International Nomenclature Cosmetic Ingredient.

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Table II
Main Composition Content for Two *Citrus unshiu* Oils

INCI name	Main composition content (%)[a]
Citrus unshiu fruit oil	dl-Limonene (92.51), gamma-Terpinene (0.68), alpha-Pinene (0.53)
Citrus unshiu peel oil	dl-Limonene (91.97), gamma-Terpinene (3.48), alpha-Pinene (0.69)

[a]Gas chromatograph mass spectrometer (GCMS-QP2010 plus, Shimadzu, Japan). INCI, International Nomenclature Cosmetic Ingredient.

METHODS

PREPARATION OF EMULSIONS

The volume of the O/W emulsion prepared was 50 mL and contained 1 wt% surfactant, 3 wt% of essential oils or HF, and 96 wt% pure water. The surfactants, sorbitan sesquioleate (HLB = 3.70) and polysorbate 60 (HLB = 14.90), comprised 1 wt% of the total blend concentration used in the O/W emulsions. A series of emulsions with HLB values were prepared by blending the emulsifiers together in different ratios (1). We can calculate the HLB of the mixed surfactants using the following formula (12,14):

$$\mathrm{HLB}_{\mathrm{mix}} = \frac{(C_a \times \mathrm{HLB}_a) + (C_b \times \mathrm{HLB}_b)}{C_{\mathrm{total}}},$$

where C_a and C_b are the quantities of surfactants (%) and HLB_a and HLB_b are the HLB values of surfactants.

After mixing the surfactant with essential oil, the mixture was kept for 2 h at a temperature control of 45°C in a screw cap bottle to prevent the essential oil from volatilization and was placed in an ultrasonic cleaner (JAC-3010, KODO, Seongnam, Korea) for 20 min and mixed well. After addition of purified water, the solution was vortexed for 1 min using a vortex mixer (vortex genie-2, Scientific Industries Inc., Bohemia, NY), and then stored at room temperature for 1 week.

Essential oil or HF 3% + Surfactant 1% (X, Y, Z) → A
↓
Put it in a 45 ℃ thermostat for 2 hours
↓
Sonication 20 min
↓
A + pure water 96%
↓
Vortexing 1 min

X: Sorbitan sesquioleate (HLB = 3.70) + polysorbate 60 (HLB = 14.90), *Y*: Sorbitan sesquioleate (HLB = 3.70) + polysorbate 20 (HLB = 16.70), *Z*: Polysorbate 60 (HLB = 14.90) + polysorbate 20 (HLB = 16.70)

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UV-VIS SPECTROPHOTOMETER MEASUREMENTS

The O/W emulsions shown previously were used for UV-vis measurements (2,4,6,15). Turbidity was compared using a UV-vis spectrophotometer (OPTIZEN Alpha, KLAB, Daejeon, Korea). For measurements, 1 mL of each CUFO and CUPO were used and diluted with 15 mL of purified water. Likewise, 1 mL of CJSO was diluted with 10 mL of purified water, and 1 mL HF was diluted with 15 mL of purified water. All measurements were carried out at 600 nm. Turbidity was measured using the following equation (1,4,15):

$$\text{Turbidity} = 100 - \%\text{T},\ \text{A} = -\log(\%\text{T}/100),$$

where T is transmittance and A is absorbance. The measurements were performed in triplicate.

TURBIDITY METER MEASUREMENT

Turbidity was compared using a turbidity meter (TU-2016, Lutron, Taipei, Taiwan). Turbidities of the essential oils or the HF emulsions were measured with the turbidity meter (Lutron) after 1 week storage at room temperature (2–4). Turbidity by nephelometry as ntu (nephelometry turbidity unit) was measured by diluting with purified water [CUFO emulsion 30% (v/v), CUPO and CJSO emulsions 50% (v/v), and HF emulsion 60% (v/v)]. Measurements were performed in triplicate.

DROPLET SIZE ANALYSIS

Oil droplet sizes of the O/W emulsions were determined by dynamic light scattering using a Nano Zeta sizer (Nano ZS, Malvern Panalytical, England). Tubes with the O/W emulsions were stored undisturbed at room temperature. On the seventh day, each emulsion was diluted using pure water (1:100). Measurements were performed in triplicate, and the average droplet size was expressed by using the mean droplet diameter.

RESULTS AND DISCUSSION

MACROSCOPIC APPEARANCE OF THE EMULSIONS

Appearance of the O/W emulsion [surfactant X: sorbitan sesquioleate (HLB = 3.70) + polysorbate 60 (HLB = 14.90)] was observed after storing for 1 week at room temperature. Figures 1, 4, and 5 show that the lower the HLB value, the more the upper part is separated. Figures 2 and 3 show that the highest turbidity when HLB had the highest value, 14.90. The comparison of the degree of emulsification by suspension or layer separation is difficult for the HF (Figure 4) and CJSO (Figure 5) emulsions in the HLB from 5.94 to 12.66.

TURBIDITY MEASUREMENT

UV-vis spectrophotometer. The O/W emulsions stored at room temperature for 1 week were used in this experiment. The turbidity values for liquid paraffin, CUFO, CUPO, HF, and CJSO emulsions at different HLB values are illustrated in Figures 6 and 7.

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Figure 1. Appearance after 1 week storage of liquid paraffin emulsions.

The highest turbidity of liquid paraffin emulsions is 63.18% when the HLB value was 10.42. The highest turbidity was shown by the CUFO (79.62%) and CUPO (76.58%) emulsions when the HLB value was 14.90. The HF emulsion displayed the maximum turbidity at 7.06 (50.77%). The CJSO emulsion showed the maximum turbidity of 73.40% when HLB was 7.06 and turbidity of 72.6% when HLB = 5.94. The CUFO and CUPO emulsions had the highest turbidity when HLB had the highest value, 14.90. Therefore, CUFO and CUPO needed additional experiments with polysorbate 20 (HLB = 16.70) so that HLB has a value higher than 14.90.

From the additional experiments, the CUFO emulsion showed the highest turbidity when HLB = 14.90 in Figure 8A and HLB = 14.75 in Figure 8B. The CUPO emulsion had the maximum turbidity at HLB = 15.35 in Figure 8A and HLB = 15.40 in Figure 8B.

To confirm whether the additional experiments using polysorbate 20 showed the same tendency as the previous experiments, the experiments were conducted using HF and CJSO emulsions. The HF emulsion had the maximum turbidity of 53.22% at an HLB value of 6.30, and CJSO emulsion had the maximum turbidity of 55.88% at an HLB value of 6.30 (Figure 9).

Figure 2. Appearance after 1 week storage of CUFO emulsions.

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Figure 3. Appearance after 1 week storage of CUPO emulsions.

Turbidity meter. The o/w emulsions stored at room temperature for 1 week were used in this experiment. The turbidity measured by the turbidity meter (Lutron) (Figures 10 and 11) showed a similar tendency to that measured by the UV-vis spectrophotometer (Figure 6). The maximum turbidities of liquid paraffin, CUFO, CUPO, HF, and CJSO emulsions were measured at HLB values of 10.42, 14.90, 14.90, 7.06, and 5.94, respectively.

Droplet size analysis. The turbidity value in Figure 10 showed the maximum value in the HLB value of the average minimum droplet size (1,2). The lowest mean droplet size of liquid paraffin (137.77 nm ± 1.46 nm), CUFO (138.00 nm ± 1.73 nm), CUPO (123.50 nm ± 3.31 nm), and CJSO (150.60 nm ± 2.88 nm) emulsions were obtained at HLB values of 14.90, 14.90, and 5.94, respectively (Figures 12 and 13).

In the method of Orafidiya and Oladimeji (1) required HLB of liquid paraffin was about 12, but it was 10.42 in this study by the analysis of mean droplet size and turbidity. This is seen as a difference in the molecular weight and structure of liquid paraffin. Meher et al. (26) reported that required HLB of light liquid paraffin was 11.80, but Niczinger et al. (2) reported required HLB of liquid paraffin as 10.0.

Figure 4. Appearance after 1 week storage of HF emulsions.

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Figure 5. Appearance after 1 week storage of CJSO emulsions.

In the case of CUFO and CUPO, required HLB was tested as 14.9 in this study, but several previous studies reported that required HLB of *D*-limonene, the main component in lemon oil, was 12.0 (2,27). However, depending on the geometrical structure of surfactants used in emulsion production, there are cases in which the required HLB for *D*-limonene was 13.7 (28) and 15.0 (29). In addition, according to Lu et al. (30), the nanoemulsion containing 10% *D*-limonene showed a minimum particle size and the maximum encapsulation rate when the HLB value was 12, but it is considered that this experiment needs to be expanded beyond HLB 12.

The lowest mean droplet size of HF emulsion (153.97 nm ± 4.23 nm) was obtained at an HLB value of 7.06 (Figure 14). From the turbidity experiment, the CUFO and CUPO emulsions had the minimum droplet size at the highest HLB value of 14.90. Therefore, the CUFO and CUPO needed additional experiments with polysorbate 20 (HLB = 16.70) to have the HLB value higher than 14.90.

Figure 6. Turbidity of liquid paraffin emulsions in the HLB range from 3.70 to 14.90, which were measured by a UV-vis spectrophotometer after 1 week storage at room temperature ($n = 3$; ±SD).

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Figure 7. Turbidities of CUFO, CUPO, HF, and CJSO emulsions in the HLB range from 3.70 to 14.90, which were measured by a UV-vis spectrophotometer after 1 week storage at room temperature ($n = 3$; ±SD).

From the additional experiments, the CUFO emulsion had a minimum droplet size (173.73 nm ± 0.43 nm) when HLB = 14.90 in Figure 15A and 127.13 nm ± 95.45 nm for the HLB value of 14.75 in Figure 15B. The CUPO emulsion had a minimum droplet size at an HLB value of 15.35 (126.73 nm ± 0.76 nm) (Figure 15A) and an HLB value of 15.40 (106.27 nm ± 4.89 nm) (Figure 15B).

To confirm whether the additional experiments using polysorbate 20 showed the same tendency as the previous experiments, the experiments were conducted using

Figure 8. Turbidities of CUFO and CUPO emulsions with (A) surfactant (polysorbate 60: polysorbate 20) HLB range 14.90–16.70, and (B) surfactant (sorbitan sesquioleate: polysorbate 20) HLB range 13.45–16.70, which were measured by a UV-vis spectrophotometer after 1 week storage at room temperature ($n = 3$; ±SD).

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Figure 9. Turbidities of HF and CJSO emulsions in HLB range 3.70–16.70, which were measured by a UV-vis spectrophotometer after 1 week storage at room temperature ($n = 3$; ±SD).

HF and CJSO emulsions. The HF emulsion had a minimum droplet size of 186.17 nm ± 0.32 nm at an HLB value of 6.30, On the other hand, the CJSO emulsion had a minimum droplet size of 188.67 nm ± 0.63 nm at an HLB value of 6.30 (Figure 16).

CONCLUSION

In the present study, we used the method of Orafidiya and Oladimeji so that the required HLB value is the HLB value when the emulsion has the maximum turbidity and the minimum droplet size during the storage period (1,2). A series of emulsions was

Figure 10. Turbidity of liquid paraffin emulsions in HLB range 3.70–14.90 which was measured by a turbidity meter after 1 week storage at room temperature ($n = 3$; ±SD).

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Figure 11. Turbidities of CUFO, CUPO, HF, and CJSO emulsions in HLB range 3.70–14.90 which was measured by a turbidity meter after 1 week storage at room temperature ($n = 3$; ±SD).

prepared by using sorbitan sesquioleate (HLB = 3.70) and polysorbate 60 (HLB = 14.90). Evaluation of turbidity using a UV-vis spectrophotometer and a turbidity meter (Lutron) showed a similar tendency. The required HLB values for maximum turbidities of CUFO, CUPO, HF, and CJSO O/W emulsions were measured as 14.90, 14.90, 7.06, and 5.94, respectively.

The CUFO and CUPO emulsions had the highest turbidity at the highest HLB value of 14.90. Therefore, CUFO and CUPO needed additional experiments with polysorbate 20 (HLB = 16.70), considering the required HLB value higher than 14.90. From the additional experiments, the CUFO emulsion exhibited the highest turbidity and minimum droplet size at HLB values of 14.75–14.90. The CUPO

Figure 12. Mean droplet size variations of emulsions of liquid paraffin in the HLB range 3.70–14.90 ($n = 3$; ±SD).

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Figure 13. Mean droplet size variations of emulsions of CUFO, CUPO, and CJSO in the HLB range 3.70–14.90 ($n = 3$; ±SD).

emulsion had the highest turbidity and minimum droplet size at HLB values of 15.35–15.40.

To confirm whether the additional experiments using polysorbate 20 shows the same tendency as the previous experiments, the experiments were conducted using HF and CJSO emulsions. The HF and CJSO emulsions had the maximum turbidity and the minimum droplet size at an HLB value of 6.30.

By measuring the turbidity [UV-vis spectrophotometer and turbidity meter (Lutron)] and droplet size (particle size analyzer) of each prepared O/W emulsion, finally the required HLB values for the CUFO, CUPO, HF, and CJSO were found to lie within 14.75–14.90, 15.35–15.40, 6.30–7.04, and 5.94–6.30, respectively.

Figure 14. Mean droplet size variation of emulsions of HF in the HLB range 3.70–14.90 ($n = 3$; ±SD).

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Figure 15. Droplet sizes of CUFO and CUPO emulsions with (A) surfactant (polysorbate 60: polysorbate 20) HLB range 14.90–16.70, and (B) surfactant (sorbitan sesquioleate: polysorbate 20) HLB range 13.45–16.70, which were measured by particle size analyzer after 1 week storage at room temperature ($n = 3$; ±SD).

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Figure 16. Mean droplet size variations of HF and CJSO emulsions in the HLB range 3.70–16.70 (sorbitan sesquioleate: polysorbate 20) after 1 week storage at room temperature ($n = 3$; ±SD).

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REFERENCES

(1) L. O. Orafidiya and F. A. Oladimeji, Determination of the required HLB values of some essential oils, *Int. J. Pharm.*, **237**, 241–249 (2002).

(2) N. A. Niczinger, N. Kállai-Szabó, J. Dredán, L. Budai, M. Hajdú, and I. Antal, Application of droplet size analysis for the determination of the required HLB of lemon oil in O/W emulsion, *Curr. Pharmaceut. Anal.*, **11**, 11–15 (2015).

(3) S. RodrÍguez-Rojo, S. Varona, M. Núñez, and M. J. Cocero, Characterization of rosemary essential oil for biodegradable emulsions, *Ind. Crop. Prod.*, **37**, 137–140 (2012).

(4) C. P. Fernandes, M. P. Mascarenhas, F. M. Zibetti, B. G. Lima, R. P. R. F. Oliveira, L. Rocha, and D. Q. Falcão, HLB value, an important parameter for the development of essential oil phytopharmaceuticals, *Braz. J. Pharmacog.*, **23**, 108–114 (2013).

(5) F. Goodarzi and S. Zendehboudi, A comprehensive review on emulsions and emulsion stability in chemical and energy industries, *Can. J. Chem. Eng.*, **97**, 281–309 (2019).

(6) M. J. Martin, L. A. Trujillo, M. C. Garcia, M. C. Alfaro, and J. Muñoz, Effect of emulsifier HLB and stabilizer addition on the physical stability of thyme essential oil emulsions, *J. Dispers. Sci. Technol.*, **39**, 1627–1634 (2018).

(7) Y. Yamashita, R. Miyahara, and K. Sakamoto, *Emulsion and Emulsification Technology, Cosmetic Science and Technology: Theoretical Principles and Applications* (Elsevier Inc., 2017), pp. 489–506, Chapter 28.

(8) N. P. Nirmal, R. Mereddy, L. Li, and Y. Sultanbawa, Formulation, characterization and antibacterial activity of lemon myrtle and anise myrtle essential oil in water nanoemulsion, *Food Chem.*, **254**, 1–7 (2018).

(9) M. Frenkel, R. Shwarts, and N. Garti, Turbidity measurements as a technique for evaluation of water-in-oil emulsion stability, *J. Dispers. Sci. Technol.*, **3**, 195–207 (1982).

(10) A. Takamura, T. Minowa, S. Noro, and T. Kudo, Effects of Tween and Span group emulsifiers on the stability of O/W emulsions, *Chem. Pharm. Bull.*, **27**, 2921–2926 (1979).

(11) A. Yaghmur, A. Aserin, Y. Mizrahi, A. Nerd, and N. Garti, Argan oil-in-water emulsions: preparation and stabilization, *J. Am. Oil Chem. Soc.*, **76**, 15–18 (1999).

(12) J. Boyd, C. Parkinson, and P. Sherman, Factors affecting emulsion stability, and the HLB concept, *J. Colloid Interf. Sci.*, **41**, 359–370 (1972).

(13) K. Koga, Y. Ishitobi, M. Iwata, M. Murakami, and S. Kawashima, A simple method for the selection of a suitable emulsifier based on color difference, *Biol. Pharm. Bull.*, **25**, 1642–1644 (2002).

(14) W. C. Griffin, Classification of surface-active agents by HLB, *J. Soc. Cosmet. Chem.*, **1**, 311–326 (1949).

(15) T.-T. Liu and T.-S. Yang, Optimization of emulsification and microencapsulation of evening primrose oil and its oxidative stability during storage by response surface methodology, *J. Food Qual.*, **34**, 64–73 (2010).

(16) J. Y. Yeon, B. R. Sin, T. G. Kim, J. M. Seo, C. H. Lee, S. G. Lee, and H. B. Pyo, A study on emulsion stability of O/W and W/S emulsion according to HLB of emulsifier, *J. Soc. Cosmet. Sci. Korea*, **40**, 227–236 (2014).

(17) M. Shahin, S. A. Hady, M. Hammad, and N. Mortada, Development of stable O/W emulsions of three different oils, *Int. J. Pharm. Stud. Res.*, **2**, 45–51 (2011).

(18) V. R. Kaufman and N. Garti, Spectral absorption measurements for determination of ease of formation and stability of oil in water emulsions, *J. Dispers. Sci. Technol.*, **2**, 475–490 (1981).

(19) R. C. Pasquali, M. P. Taurozzi, and C. Bregni, Some considerations about the hydrophilic-lipophilic balance system, *Int. J. Pharm.*, **356**, 44–51 (2008).

(20) L. K. Hong, S. I. Kim, and S. B. Lee, Effects of HLB value on oil-in-water emulsion: droplet size rheological behavior, zeta-potential, and creaming index, *J. Ind. Eng. Chem.*, **67**, 123–131 (2018).

(21) A. R. Lee and E. Yi, Bioactive properties of *Citrus unshiu*'s essential oil for its application to textiles, *Fibers Polym.*, **14**, 1295–1300 (2013).

(22) X. N. Yang and S. C. Kang, Chemical composition, antioxidant and antibacterial activities of essential oil from Korean *Citrus unshiu* peel, *J. Agric. Chem. Environ.*, **2**, 42–49 (2013).

(23) E. Jung, J. Lee, J. Baek, J. Jung, J. Lee, S. Huh, S. Kim, J. Koh, and D. Park, Effect of *Camellia japonica* oil on human type I procollagen production and skin barrier function, *J. Ethnopharmacol.*, **112**, 127–131 (2007).

(24) T. Satou, N. Sato, H. Kato, M. Kawamura, S. Watanabe, and K. Koike, The effect of *Camellia* seed oil intake on lipid metabolism in mice, *Nat. Prod. Commun.*, **11**, 511–513 (2016).

(25) Y. S. Lee, J.-H. Yoon, B.-A. Kim, C. I. Park, W. K. Yoo, J. W. Cho, and M. R. Kim, Effects of horse oil on the DNCB-induced contact hypersensitivity, *Korea J. Herbo.*, **28**, 77–81 (2013).

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(26) J. G. Meher, N. P. Yadav, J. J. Sahu, and P. Sinha, Determination of required hydrophilic–lipophilic balance of citronella oil and development of stable cream formulation, *Drug Dev. Ind. Pharm.*, **39**, 1540–1546 (2013).
(27) W.-C. Lu, T.-J. Zhang, D.-W. Huang, and P.-H. Li, Nanoemulsion of D-limonene in water system prepared by ultrasonic emulsification, *J. Cosmet. Sci.*, **65**, 245–252 (2014).
(28) J. Yang, W. Jiang, B. Guan, X. Qiu, and Y. Lu, Preparation of D-limonene oil-in-water nanoemulsion from an optimum formulation, *J. Oleo Sci.*, **63**, 1133–1140 (2014).
(29) M. R. Zahi, P. Wan, H. Liang, and Q. Yuan, Formation and stability of D-limonene organogel-based nanoemulsion prepared by a high-pressure homogenizer, *J. Agric. Food Chem.*, **62**, 12563–12569 (2014).
(30) W.-C. Lu, B.-H. Chiang, D.-W. Huang, and P.-H. Li, Skin permeation of D-limonene-based nanoemulsions as a transdermal carrier prepared by ultrasonic emulsification, *Ultrason. Sonochem.*, **21**, 826–832 (2014).

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