Food Chemistry 272 (2019) 404–410

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

UV and storage stability of retinol contained in oil-in-water nanoemulsions

Heesoo Park, Saehun Mun*,[1], Yong-Ro Kim*,[1]

Center for Food and Bioconvergence, and Department of Biosystems and Biomaterials Science and Engineering, Seoul National University, Seoul 08826, Republic of Korea

ARTICLE INFO

Keywords:
Retinol
Emulsion
UV stability
Storage stability
Lipid oxidation

ABSTRACT

This study was performed to examine the stability of retinol contained within oil-in-water (O/W) emulsions under UV and during storage at different temperatures. O/W emulsions were prepared using different emulsifiers and oil concentrations. The stability of the retinol contained in the O/W emulsions was investigated by measuring the percentage of residual retinol in the samples after UV exposure and storage at different temperatures (4, 25, and 40 °C). The oil concentration of the emulsion had a greater impact on UV stability than the type of emulsifier used, whereas the storage stability at different temperatures was affected by both the choice of emulsifier and the oil concentration. The storage stability of the retinol contained in the O/W emulsions may be related to the lipid oxidation properties of the emulsions rather than the latter's physical stability. Experiments with EDTA and different oil types were performed to confirm this theory.

1. Introduction

All-trans retinol (AR) is a hydrophobic vitamin A compound that is essential for animal growth, proper functioning of the optical transduction system and the immune system, and differentiation of epithelial tissue (Eskandar, Simovic, & Prestidge, 2009a,b; Sauvant, Cansell, Sassi, & Atgié, 2012).

Many studies have been performed to improve the stability of retinol, especially for use in pharmaceutical and cosmetic formulations (Asai & Watanabe, 2000; Jenning & Gohla, 2001; Ghouchi-Eskandar, Simovic, & Prestidge, 2012; Yanaki, 2001). However, little research has been done in the food sector. Retinol deficiency can cause severe health problems because retinol plays an important role in many of the body's physiological processes. Many children in developing countries still lose their sight and die from a lack of retinol. The primary cause of vitamin A deficiency is insufficient dietary intake, which can be corrected by consumption of vitamin A-rich foods. However, retinol, with *trans* double bonds in the isoprenoid side chain, is easily degraded as it is sensitive to oxygen, heat, and light, and degrades through various instability pathways such as isomerization to cis-isomers, molecular fragmentation, and photochemical oxidation–reduction (Lee et al., 2002; Gonçalves, Estevinho, & Rocha, 2016). In addition, it is difficult to incorporate retinol directly into food products because retinol may crystallize at room temperature and has low water solubility. Therefore, many studies have worked on developing formulations and delivery systems that improve the incorporation of retinol into food and increase its chemical stability in food products (Eskandar et al., 2009a,b; Jenning & Gohla, 2001; Lee et al., 2002).

A number of delivery vehicles for retinols have been developed, including liposomes, nanoemulsions, solid lipid nanoparticles, formation of complexes, and nanoparticle coated emulsions (Eskandar et al., 2009a,b; Ghouchi-Eskandar, et al., 2012; Jenning & Gohla, 2001; Lee et al., 2002; Qi & Shieh, 2002; Yoshida, Sekine, Matsuzaki, Yanaki, & Yamaguchi, 1999). For food applications the retinol delivery system must fulfil specific functions, such as stabilizing retinol, preventing its degradation during food processing and storage, and minimizing the effects of reactive species on the system, whilst not modifying the sensory properties of the food product.

Yoshida et al. (1999) loaded retinol into three types of emulsions, oil-in-water (O/W), water-in-oil (W/O), and oil-in-water-in-oil (O/W/O), and reported that the stability of retinol in the O/W/O was the highest amongst the three types of emulsions, suggesting that O/W/O emulsions are useful formulae for stabilizing vitamin A (Yoshida et al., 1999). Another study investigated the chemical stability and phase distribution of AR in nanoparticle-coated emulsions and reported that the chemical stability of AR in this system was linked to the phase distribution of the active agent and the interfacial structure of the emulsions (Eskandar et al., 2009a). In addition, a study revealed that the aqueous solubility of all-trans retinoic acid (ATRA) increased when ATRA formed a complex with β-cyclodextrins (Qi & Shieh, 2002).

In this study, retinol-loaded O/W nanoemulsions were prepared and their characteristics were investigated. It is the first step in developing a

* Corresponding authors.
E-mail addresses: sdbsm7@snu.ac.kr (H. Park), saehun@snu.ac.kr (S. Mun), yongro@snu.ac.kr (Y.-R. Kim).
[1] These authors contributed equally.

https://doi.org/10.1016/j.foodchem.2018.08.076
Received 23 March 2018; Received in revised form 1 August 2018; Accepted 19 August 2018
Available online 20 August 2018

retinol delivery system applicable to food products, as O/W emulsions are one of the delivery systems that can be easily and economically manufactured. This paper also presents the effects of emulsifiers (Tween 20, decaglycerin myristate, and whey protein isolate) and oil concentrations on the UV and storage stability of retinol contained in O/W emulsions. It is well known that the emulsifier is an important factor for manufacturing the stable emulsion systems. Some of emulsifiers such as polysorbate and glycerol esters are solely emulsifiers, which are called small molecule emulsifiers, while some of them such as milk proteins and modified starches have both emulsifying and stabilizing properties, which are called as large molecule emulsifiers. Therefore, two types of emulsifiers were used to prepare emulsion in current study (Mao et al., 2009). Tween 20 and decaglycerin myristate were used as small molecule emulsifier and whey protein isolate was used as large molecule emulsifiers.

2. Materials and methods

2.1. Materials

AR and Tween 20 were purchased from Sigma Aldrich (St. Louis, MO, USA). Chemical Co. Whey protein isolate (WPI; Product code: 9500) was obtained from Protient, Inc. (St. Paul, MN, USA). Decaglycerin myristate (DM) was obtained from Mitsubishi-kagaku Foods Corporation (Shinba-koen, Minato-ku, Tokyo, Japan). Soybean oil and coconut oil were purchased from a local supermarket and used without further purification. All other chemicals were of analytical grade.

2.2. Preparation of retinol-loaded O/W nanoemulsions

To ensure the same amount of retinol was added to the emulsions prepared with different oil concentrations, 5, 1, 0.5, 0.25, 0.125 and 0.05 wt% of retinol was directly added to 0.1, 0.5, 1, 2, 4 and 10 g of oil, respectively, to obtain O/W nanoemulsions containing 0.1, 0.5, 1, 2, 4 and 10 wt% oil, respectively. The retinol-containing soybean oil was sonicated (Ultrasonic cleaner-Powersonic 410, Hwashin, Seoul, Korea) for 15 min to ensure complete retinol dissolution. Three emulsifiers (Tween 20, DM, and WPI) were used to investigate the effect of emulsifier type on the stability of retinol contained in O/W nanoemulsions. Emulsifier solutions were prepared by adding each emulsifier to 10 mM phosphate buffer (pH 7) and gently stirring for 3 h. The used ratio of oil to emulsifier of prepared emulsions was 6:1. Coarse emulsions were prepared by mixing combinations of retinol-containing oils and emulsifier solutions using a high speed blender (ULTRA-TURRAX model T25 digital, IKA, Germany) for 1 min at 12,000 rpm. Then nanosized emulsions were prepared by passing the solution through a microfluidizer (Picomax MN 250A, Micronox, Seongnam, Korea) three times at 0.5 MPa. All samples were stored at 4 °C.

In addition, to check the zeta-potential value of retinol-loaded O/W nanoemulsion when the amount of retinol contained in oil is different, retinol-loaded Tween 20-stabilized emulsions were prepared with a fixed oil concentration of 4 wt% and varying amounts of retinol (1.25, 2.5, and 5 mg). The following procedures were the same as the above.

2.3. Characteristics of the emulsions

The droplet size, size distribution and zeta-potential of the emulsions were analyzed by the dynamic light scattering method using Zetasizer (Zetasizer Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK). Prior to analysis, the emulsions were diluted with 10 mM phosphate buffer (pH 7) to avoid multiple scattering. Mean droplet size (Z-average) was obtained from the graph of single intensity.

After diluting the emulsion 100-fold, the turbidity of the emulsions was measured with a UV spectrophotometer (UV-1800, SHIMADZU, Japan) at 500 nm.

2.4. UV stability of retinol in O/W nanoemulsions

The UV stability of retinol loaded in O/W nanoemulsions was investigated using a UVA irradiation chamber. Each sample (4 mL) was placed on a rotating plate at a distance of about 10 cm from the G8T5 UV lamp (8 W; Philips, Poland) and irradiated with UV for up to 24 h.

The concentration of retinol remaining in the emulsions was determined using spectrophotometric analysis.

2.5. Storage stability of retinol in O/W nanoemulsions

To evaluate the storage stability of retinol loaded in O/W nanoemulsions, each prepared emulsion (1 mL) was added into a 1.5 mL brown tube and stored at 4, 25 or 40 °C. The amount of retinol remaining was measured for 1 week. Samples were equilibrated at room temperature for 10 min before analysis. Bulk oil containing retinol was used for comparison. The remaining concentration of retinol was determined using spectrophotometric analysis.

2.6. Retinol content analysis

The retinol content was determined using the method published by Zhao et al. (Zhao, Guan, Pan, Nitin, & Tikekar, 2015) with slight modification. To extract the retinol contained in the oil and emulsions, 100 μL of each sample was mixed with 1900 μL of methanol (20 times dilution) using a vortex mixer for 1 min. Then the mixture was centrifuged (Supra-22K, HANIL, Korea) at 14,000 rpm for 10 min and the supernatant was filtered using a 0.45 μm filter (Part no. 89713, TPP, Switzerland). The absorbance of the retinol in the filtered supernatants was measured at 325 nm. Blank samples containing emulsions without retinol were also tested. The absorbance was measured using a UV spectrophotometer (UV-1800 model, Shimadzu, Japan). The standard curve ($R^2 = 0.998$) was prepared by measuring the absorbance of known concentrations of retinol in methanol.

2.7. Effect of EDTA and oil type on retinol stability

To determine the effect of EDTA on retinol stability, EDTA (75 mM) was added to retinol-loaded O/W nanoemulsions prepared with WPI and 4 wt% oil and prepared emulsions were stored at 4, 25, and 40 °C for one week. In the case of the effect of oil type on retinol stability, it was examined by comparing the residual retinol content from soybean oil emulsion to that from coconut oil emulsion. The % residual retinol was determined as described above.

2.8. Statistical analysis

All data were analyzed using SPSS for Windows (version 24.0; SPSS inc., Chicago, IL, USA). The one-way ANOVA test was performed followed by Student-Newman-Keuls's multiple range tests. All data are reported as means ± standard deviations. A p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Characterization of retinol-loaded O/W nanoemulsions

3.1.1. Effect of the emulsifier type

The retinol-loaded O/W nanoemulsions were prepared with Tween 20, DM and WPI, and the characteristics of the prepared emulsions were examined by measuring droplet size and zeta-potential (Fig. 1). The zeta-potential of the emulsions stabilized by Tween 20, DM and WPI were around −10.3 ± 1.5, −13.4 ± 2.1, and −44.6 ± 1.1 mV, respectively. WPI-stabilized emulsion droplets were highly negative compared to those stabilized by Tween 20 and DM because the emulsions were prepared at pH 7, which is appreciably above the isoelectric

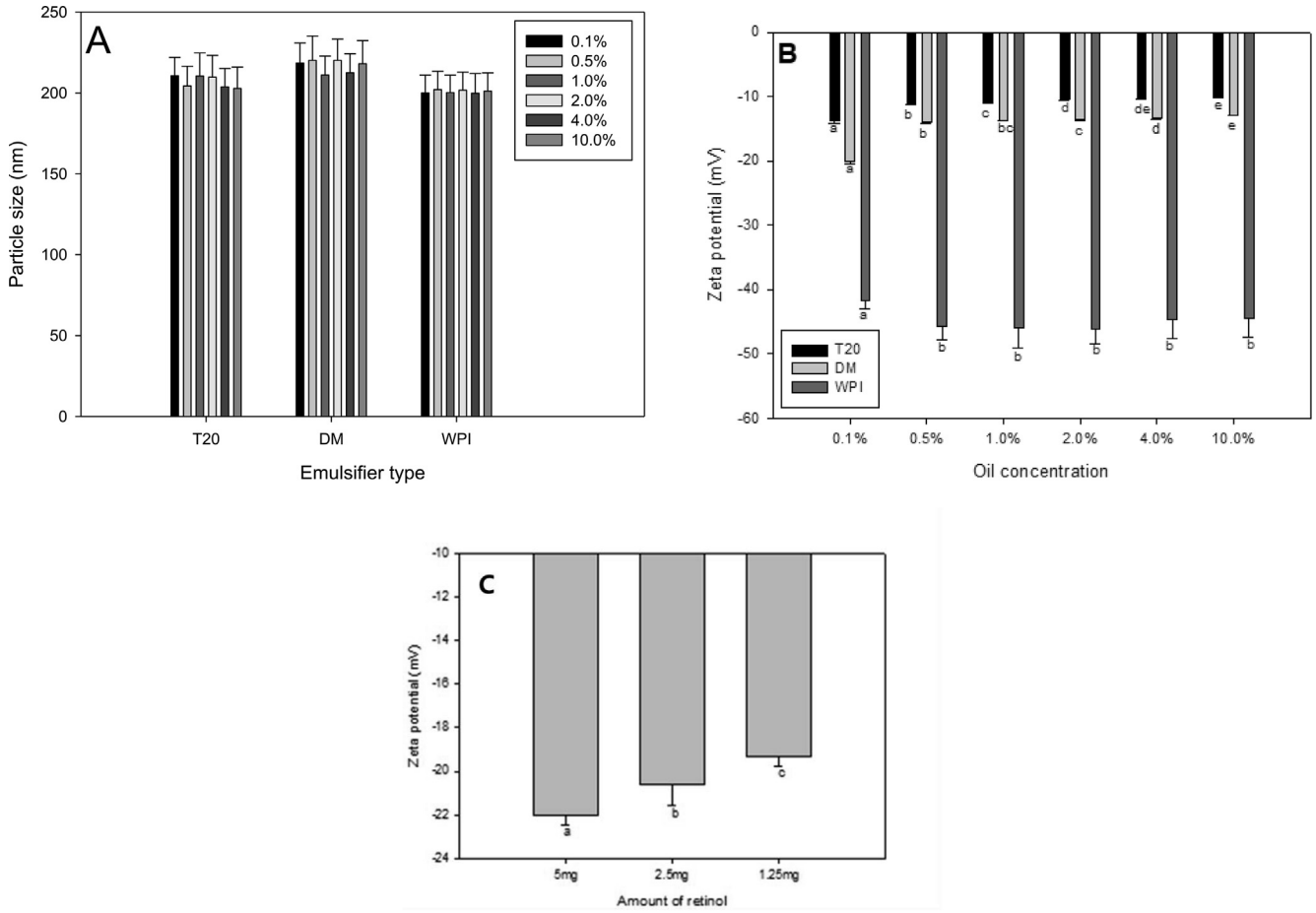

Fig. 1. Mean droplet size (A) and zeta-potential of retinol-loaded O/W emulsions (B) prepared with different emulsifiers. For measuring size and zeta-potential, emulsions prepared with 0.1, 0.5, 1.0, 2.0, 4.0 and 10.0% oil were used. Different lower case mean significant difference ($p < 0.05$) on the zeta-potential between emulsions with different oil concentrations. T20; Tween 20, DM; Decaglycerin myristate, WPI; Whey protein isolate. Zeta potential value (C) of retinol-loaded O/W emulsion (4% oil emulsion using Tween20 as an emulsifier) when the amount of retinol contained in oil is different (1.25, 2.5 and 5 mg in 4 g oil). Different lower case mean significant difference ($p < 0.05$).

point of WPI (p*I* ~ 5). Even though Tween 20 and DM are non-ionic emulsifiers, the emulsion droplets containing these emulsifiers had an appreciable negative charge. Previous studies have reported adsorption of impurities or of OH– species from water to the oil-water interface (McClements, 2005; Marinova et al., 1996; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013), which could explain this result.

The mean droplet sizes of the emulsions stabilized with Tween 20, DM, and WPI were around 200 nm and there were no significant differences between the different emulsions (Fig. 1(A)). Droplet size distributions were monomodal showing a single peak (data not shown) and phase separation of retinol was not observed from any of the emulsions. Therefore, these results suggest that stable retinol-loaded emulsions were prepared using the method presented in this study and that the choice of emulsifier did not significantly affect the characteristics of the emulsions.

3.1.2. Effect of the oil concentration

Emulsions with different oil concentrations but equal amounts of retinol were prepared. The oil concentration did not significantly affect the mean droplet size or droplet distribution. However, the zeta-potential value did vary depending on the emulsion's oil concentration (Fig. 1(B)). As the oil concentration increased, the absolute value of the zeta-potential decreased in Tween 20 and DM stabilized emulsions. This result may be due to the amount of retinol present in the oil. According to previous studies, the amphiphilic AR may adsorb to the oil–water interface by means of the hydroxyl group in its isoprenoid side chain (Eskandar et al., 2009a). Hence, the lower the oil concentration, the higher the relative amount of retinol present in the oil, and the more retinol present at the interface resulting in an increased negative zeta-potential value. This effect was strong in Tween 20 and DM-stabilized emulsions, but was negligible in WPI-stabilized emulsion.

In order to confirm this theory, retinol-loaded Tween 20-stabilized emulsions were prepared with a fixed oil concentration of 4 wt% and varying amounts of retinol (1.25, 2.5, and 5 mg). The results show that as the amount of retinol in the oil increased, so did the negative value of the zeta-potential (Fig. 1(C)). The zeta potential is a measure of the charge on the surface of the oil droplet. Therefore, this result indicates that the change in value might be caused by retinol dispersing on the surface of the oil rather than within the oil, thus affecting the surface charge. In a previous study, it was found that when α-tocopherol was encapsulated in the lipid droplets of an O/W emulsion, its hydrophilic group was in the continuous phase, whereas its lipophilic tail was in the oil; hence, α-tocopherol was distributed on the surface of the oil droplets (Silvestre, Chaiyasit, Brannan, McClements, & Decker, 2000). Similarly, retinol is mainly hydrophobic with the exception of a single hydroxyl group that prevents it from completely dispersing in oil. These observations are related to the UV and storage stability of the retinol contained in the investigated O/W emulsion systems, which will be explained later in this paper.

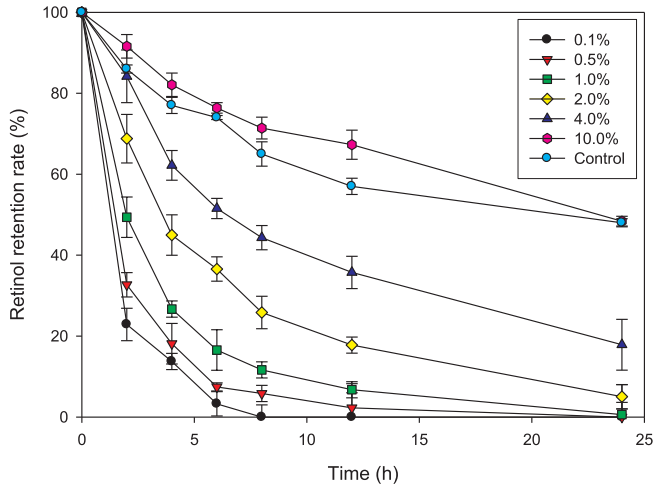

Fig. 2. UV-stability of retinol incorporated in O/W emulsions prepared with decaglycerin myristate as an emulsifier and different concentration of oil (0.1, 0.5, 1, 2, 4 and 10%).

3.2. UV stability of retinol in O/W nanoemulsions

The UV stability of retinol incorporated into the oil phase of emulsions was investigated by measuring the % residual retinol after sample exposure to UV (Fig. 2). The % residual retinol was measured at various time points over 24 h and the UV stability of the retinol contained in the emulsions was compared to that of retinol contained in bulk oil (control). The results show that as the emulsion's oil concentration increased, so did the UV stability of the retinol it contained. However, when comparing the UV stability of the retinol contained in the emulsions to that of the retinol dispersed in bulk oil, it was seen that at oil concentrations below 10 wt%, the retinol retention rate of the emulsions was lower than that of the bulk oil. However, at 10 wt% oil, the retinol retention rate of the emulsions became greater than that of the bulk oil. This result suggests that the O/W nanoemulsions systems with low oil concentrations, irrespective of the emulsifier used (Tween 20, DM, or WPI), are not effective at protecting retinol against UV; however, over a certain oil concentration, they become effective.

Emulsions form oil droplets, therefore, a larger lipid surface area is exposed to the water phase and light than with bulk oil; thus, more light can penetrate into the emulsions. Furthermore, as previously mentioned, retinol is likely to be dispersed on the surface of the oil rather than entirely within the oil. Thus, the retinol that is contained within the emulsion systems is more easily reached and degraded by UV compared to retinol dispersed in bulk oil.

Eskandar et al. (2009a) reported that despite the relatively low water solubility of retinol at room temperature and pH 7.3, retinol can move through the aqueous phase, thus, resulting in the fast partitioning of AR from the oil phase of the emulsions to the aqueous phase. They also suggested that this kind of distribution of AR could be limited by adding an interfacial barrier such as a silica-oleylamine complex at the oil–water interface (Eskandar et al., 2009a). Cho, Cho, Choi, and Cheong (2012) prepared the Retinol 50C emulsion, which is co-stabilized with triblock copolymers. They suggested that the UV stability of retinol was improved by using triblock copolymers with a long PCL (polycaprolactone) block length and low HLB value (Cho et al., 2012). These previous studies suggest that the structure of the oil–water interface and the interaction of retinol with the emulsifier at the interface may be another important factors to protect the retinol loaded in O/W emulsions from UV degradation. Therefore, we deduced that the emulsifiers used in this study were not effective for retinol partitioning at lower oil concentrations.

The higher UV stability of retinol contained in emulsions with 10 wt % oil was likely attributable to the turbidity of these emulsions because more lipid droplets could scatter light. We hypothesized that differences in the ability of UV to penetrate the emulsions affected the UV stability. The turbidity of the emulsions was measured to prove this hypothesis. Measured turbidity values of emulsions containing 0.1, 0.5, 1.0, 2.0, 4.0, and 10 wt% oil were 0.069 ± 0.002, 0.159 ± 0.012, 0.344 ± 0.014, 0.555 ± 0.024, 0.799 ± 0.006, and 1.497 ± 0.02, respectively. Emulsions prepared with lower oil concentrations were more transparent. As the oil concentration in the emulsions increased so did the turbidity, suggesting that the turbidity of O/W nanoemulsions can affect the UV stability of the retinol contained in the emulsions.

3.3. Storage stability of retinol at different temperatures

The effect of storage temperature (4, 25 and 40 °C) on the stability of the retinol contained in the emulsions prepared with different oil concentrations and emulsifiers was examined.

First, the stability of the emulsions themselves was examined by measuring the mean droplet size and zeta-potential over time. The mean droplet size of freshly prepared emulsions with different oil concentrations and emulsifiers was not significantly different (Fig. 1(A)), however, when the emulsions were stored at different temperatures, the droplet size and appearance of the emulsions changed depending on the oil concentration and storage temperature (Fig. 3). At 4 °C and 25 °C, the mean droplet size was not affected.

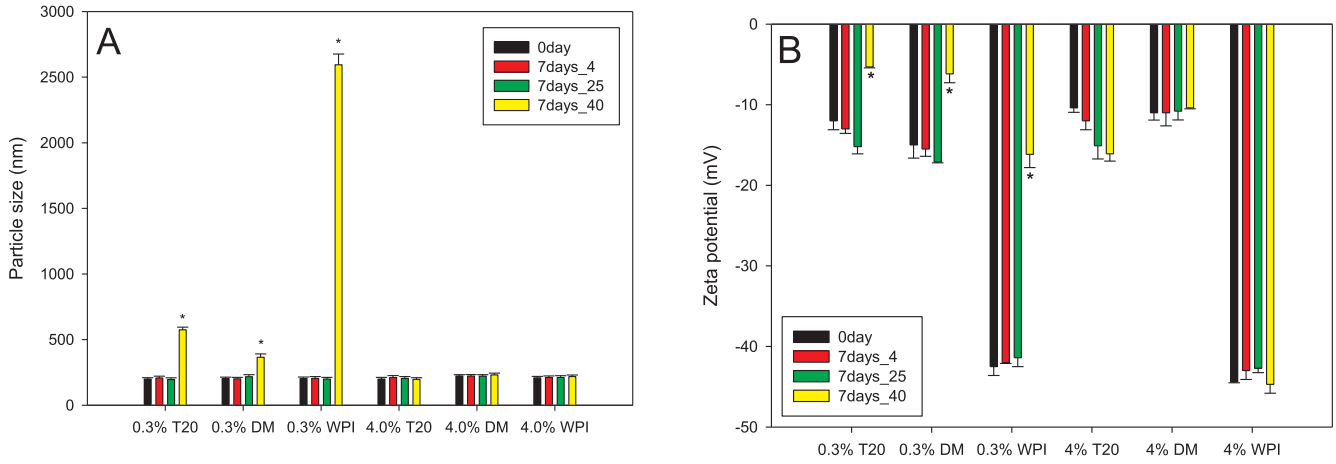

Fig. 3. Changes in the particle size (A) and zeta potential value (B) of O/W emulsions stored at different temperature (4, 25 and 40 °C) and for one week. Emulsions were fabricated with different oil concentrations (0.3, 4% oil emulsion) and different emulsifiers (Tween 20 (T20), Decaglycerin myristate (DM) and WPI). * means significant difference ($p < 0.05$) on the value between emulsions stored at different storage temperatures.

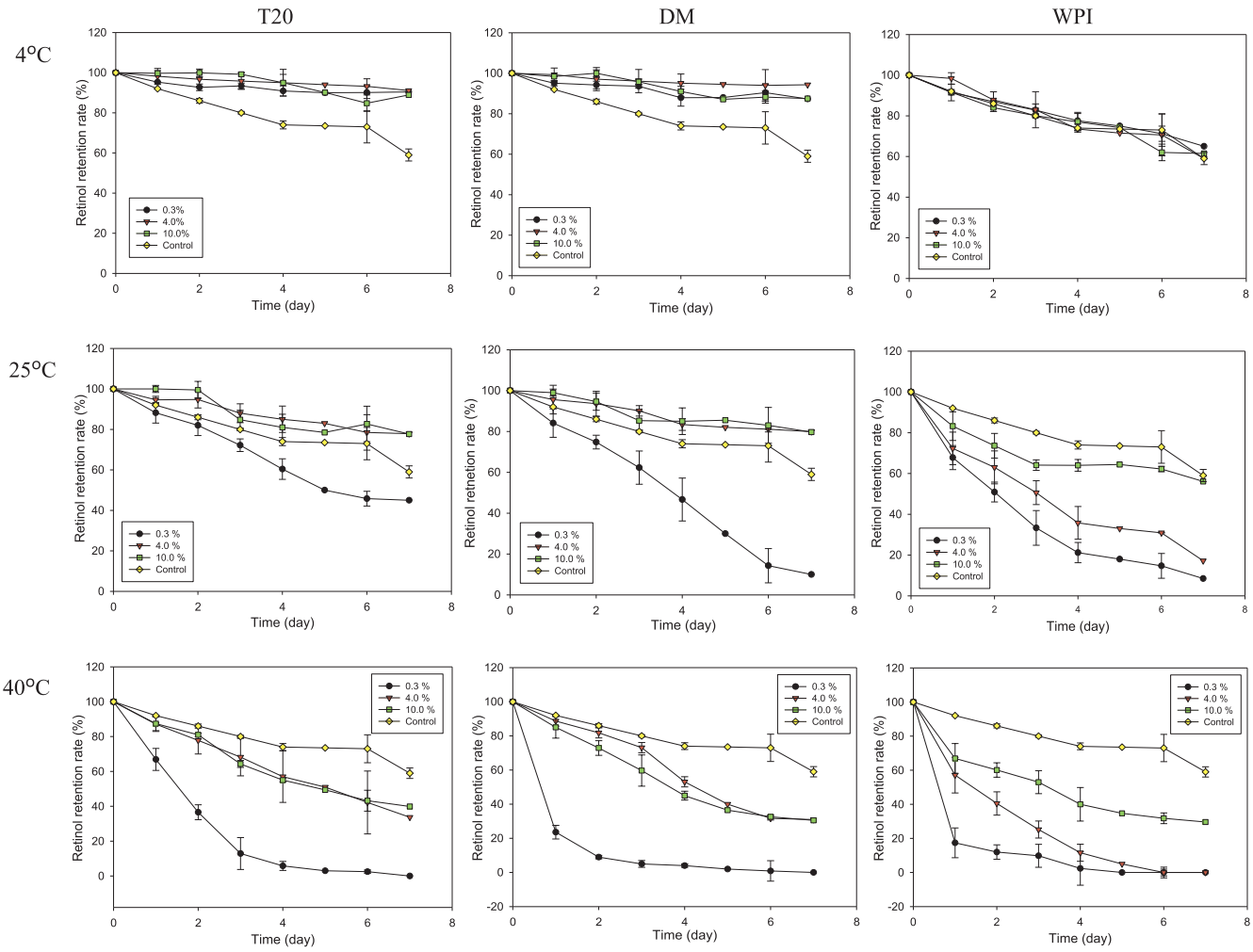

Fig. 4. Retention rate (%) of retinol in O/W emulsion according to oil concentration (0.3, 4 and 10% w/w), type of emulsifier (Tween 20 (T20), Decaglycerin myristate (DM) and WPI) and storage temperature (4, 25 and 40 °C).

However, at 40 °C a relationship between the size of the emulsion droplets and the oil concentration of emulsions was observed. At a relatively lower oil concentration (0.3 wt% oil), the size of the emulsion droplets increased over time and the emulsions were unstable regardless of the type of emulsifier used, whereas, the mean droplet size and size distribution (data not shown) were kept constant at a higher oil concentration (4 wt%). The zeta-potential values confirmed these results. When the emulsions prepared with 0.3 wt% oil were stored at 40 °C, the value of the negative charge decreased indicating that the repulsive forces between the oil droplets became weaker.

The storage stability of the retinol contained in the emulsions was investigated by measuring the % residual retinol throughout 1 week of storage at different temperatures (Fig. 4). At a storage temperature of 4 °C, the retinol concentration in the Tween 20- and DM-stabilized emulsions was maintained regardless of the oil concentration. However, the % residual retinol in the WPI-stabilized emulsions decreased in a manner similar to the retinol contained in the bulk oil. When samples were stored at 25 °C, the % residual retinol was dependent on the oil concentration of the emulsions. After 1 week of storage, the % residual retinol in Tween 20- and DM-stabilized emulsions with higher oil concentrations (4 and 10 wt%) was around 80%, which was higher than in the bulk oil. However, lower residual amounts of retinol were found in the emulsions stabilized with WPI than in the bulk oil at this storage temperature, even though the droplet size of the WPI emulsions did not change over time. At a storage temperature of 40 °C, none of the emulsion systems were able to prevent retinol from degrading during storage, and the rate of decomposition increased as the oil concentration decreased.

We expected that the stability of the emulsions themselves would be related to retinol stability during storage, however, the results suggest that the ability of the emulsions to stabilize retinol over long periods of storage is not related to the physical stability of the emulsions themselves. The mean droplet size of the emulsions prepared with a higher oil concentration (4 wt%) was not changed after storage regardless of the temperature or the type of emulsifier (Fig. 3). However, at high temperatures, the stability of the retinol contained in the emulsions was lower than that of the retinol in bulk oil. The retinol retention rate of the retinol-loaded WPI-stabilized emulsions was lower than that of bulk oil at all temperatures. This may be caused by lipid oxidation. It is well known that the rate of oxidation is significantly enhanced in O/W emulsion systems compared to bulk food products because of the large lipid surface area and preferential localization of lipid hydroperoxides at the oil–water interface (Chaudhari & Nitin, 2015; McClements & Decker, 2000).

Although the % residual retinol of the emulsion systems decreased over time, around 80% of the retinol was maintained in the higher oil concentration Tween 20- and DM-stabilized emulsions at storage temperatures of 4 and 25 °C. However, the % residual retinol in the WPI-stabilized emulsion was constantly lower than that in the bulk oil. The type of emulsifier used can affect lipid and retinol oxidation. Tween 20 and DM are nonionic emulsifiers, whereas WPI is anionic emulsifier. Previous studies have reported that anionic emulsifiers promote lipid

oxidation within emulsion systems (Hu, McClements, & Decker, 2003; McClements & Decker, 2000). They reported that the lipid oxidation rates were highest for negatively charged droplets, whereas they were fairly lower and similar for uncharged droplets and positively charged surface. Negatively charged droplets can easily react with trace amounts of metal ions present in the aqueous phase. This may explain the greater degradation seen with WPI. Another factor is the effect of the aqueous phase pH on the trace amounts of iron ions in the water. For example, at pH 7, the solubility of iron ions is low so they are not dissolve in the aqueous phase but rather exist at the oil–water interface; thus, oxidation is promoted on the surface of the oil droplets (Donnelly, Decker, & McClements, 1998). Moreover, in this study the emulsions were prepared at pH 7, which is higher than the p*I* of WPI, so within our emulsion systems WPI was negatively charged. As mentioned previously, retinol was distributed at the oil–water interface of the emulsions and within the oil, therefore, it is likely that the retinol contained in the emulsions stabilized with WPI was exposed to conditions that would cause it to be more easily oxidized.

3.4. Effect of EDTA and oil type on retinol stability

In order to confirm whether the interpretations given above are correct, EDTA, which chelates metals, was added to the retinol-loaded O/W nanoemulsions prepared with WPI. The % residual retinol are shown in Fig. 5. At a storage temperature of 4 °C, the % residual retinol in the emulsion with EDTA became higher than in that without EDTA after 3 days. However, at 25 and 40 °C, the % residual retinol was higher in the emulsion containing EDTA than in that without EDTA throughout the 7 days, suggesting that WPI reacts with the metal ions present in the aqueous phase promoting the oxidation of retinol.

Finally, the effect of the oil type on the stability of the retinol contained in O/W nanoemulsions was examined. If lipid oxidation was responsible for retinol degradation, we hypothesized that retinol-loaded emulsions prepared with different oils that have different lipid oxidation rates, would show different retinol retention rates. In this study, the oil used to make the O/W emulsions was soybean oil, which is a long-chain triglyceride containing about 55% linoleic acid, and which is vulnerable to oxidation. Alternatively, coconut oil composed of 95% medium-chain triglyceride was used to prepare emulsions (Kupongsak & Sathitvorapojjana, 2017).

The stability of retinol was higher in emulsions prepared using coconut oil (Fig. 5). The retention rate of retinol in WPI-stabilized emulsions was similar to that of bulk oil at 4 °C but lower at 25 and 40 °C. However, when the soybean oil was replaced with coconut oil, the retention rate of retinol improved at all of temperatures. These results are due to the different compositions of fatty acids of the two kinds of oils. Oxidation is promoted as the number of double bonds increases. In other words, the higher the content of unsaturated fatty acids, the more easily the oxidation proceeds. Soybean oil has about 15% saturated fatty acid and about 80% unsaturated fatty acid, whereas coconut oil has only 9% unsaturated fatty acid (Kupongsak & Sathitvorapojjana, 2017). Therefore, the oxidation rate of the oil used in the emulsion has an effect on the oxidation of the retinol distributed at the oil's surface.

From these results, we concluded that the stability of the retinol incorporated into WPI-stabilized emulsions was especially lower than that of retinol contained in other emulsions or bulk oil because of the greater lipid oxidation in these emulsions. It was also seen that retinol stability was not dependent on the physical stability of the emulsions themselves, but on the oxidation properties of the emulsion systems, which could be affected by various factors, such as the type of emulsifier used, the unsaturated fatty acid content, the pH, etc. Ultimately affecting the rates of retinol oxidative degradation.

4. Conclusions

The stability of retinol contained within O/W nanoemulsions under UV and during storage at different temperatures was examined. After exposure to UV, the % residual retinol was lower in emulsions systems prepared with low oil concentrations than in bulk oil. However, above 10 wt% oil concentration, the % residual retinol was greater than in the bulk oil because of the higher turbidity of the emulsions. This result can be rationalized using the following explanations: i) emulsion systems have larger lipid surface areas exposed to the aqueous phase and more

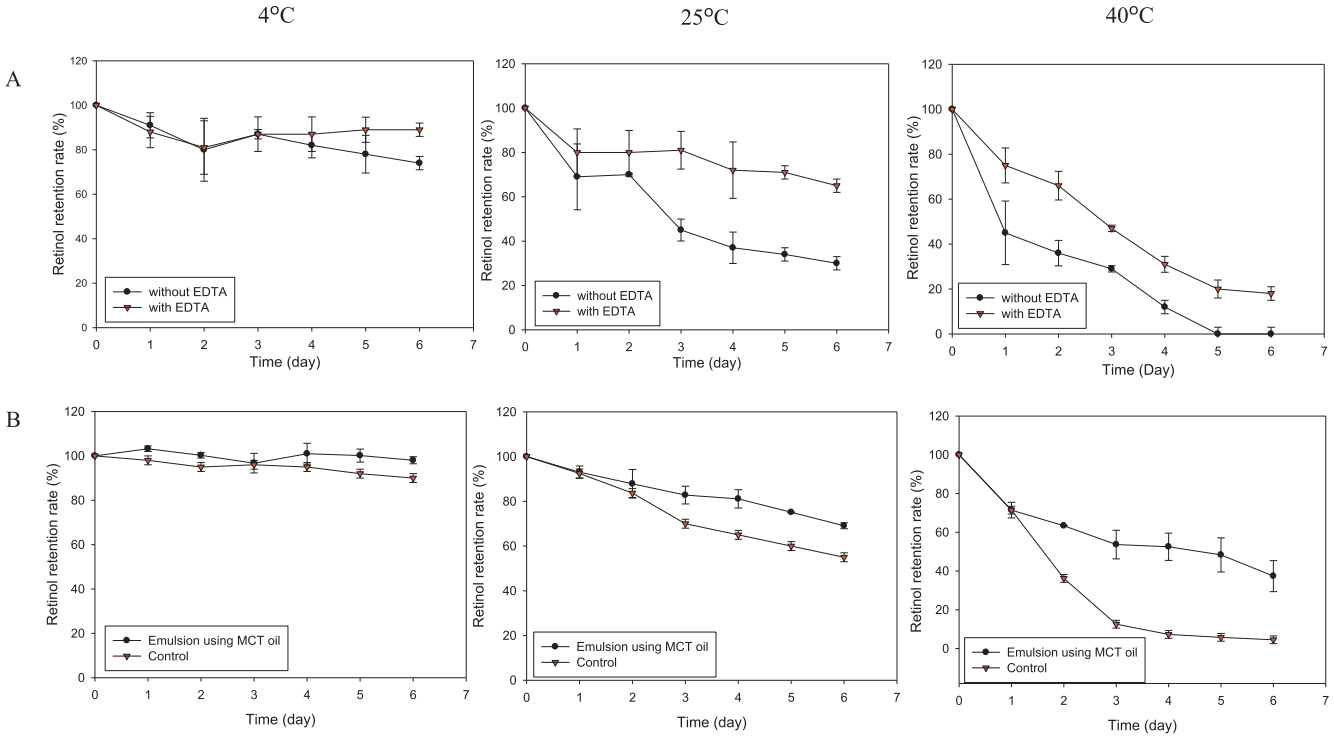

Fig. 5. Retention rate (%) of retinol in O/W emulsion (soybean oil 4% w/w) stabilized with WPI. (A) Effect of EDTA, (B) Effect of oil type.

light can penetrate through the emulsions than the bulk oil, ii) as shown by the zeta-potential measurements, within the emulsions retinol is dispersed on the surface of the oil, suggesting that the degradation of retinol occurs at the water-oil interface.

The results obtained from storage experiments of retinol-loaded O/W nanoemulsions suggested that the long-term stability of retinol incorporated into O/W nanoemulsions might be related to the chemical stability of the emulsions rather than their physical stability. This means that although the emulsions themselves were physically stable, some systems such as the WPI-stabilized emulsions still had poor retinol retention rates, possibly due the presence of factors that promote oxidation within these systems.

Acknowledgements

This work was partly supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High value-added Food Technology Development Program (Project No. 315065-3), funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea. This work was also partly supported by Youlchon Foundation (Republic of Korea).

Conflict of interest

The authors declared no conflict of interest.

References

Asai, Y., & Watanabe, S. (2000). Formation and stability of the dispersed particles composed of retinyl palmitate and phosphatidylcholine. *Pharmaceutical Development and Technology, 5*, 39–45.

Chaudhari, A., & Nitin, N. (2015). Role of oxygen scavengers in limiting oxygen permeation into emulsions and improving stability of encapsulated retinol. *Journal of Food Engineering, 157*, 7–13.

Cho, H. K., Cho, J. H., Choi, S. W., & Cheong, I. W. (2012). Topical delivery of retinol emulsions co-stabilised by PEO-PCL-PEO triblock copolymers: Effect of PCL block length. *Journal of Microencapsulation, 29*(8), 739–746.

Donnelly, J., Decker, E., & McClements, D. (1998). Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. *Journal of Food Science, 63*(6), 997–1000.

Eskandar, N. G., Simovic, S., & Prestidge, C. A. (2009b). Nanoparticle coated submicron emulsions: Sustained in-vitro release and improved dermal delivery of all-trans-retinol. *Pharmaceutical Research, 26*(7), 1764–1775.

Eskandar, N. G., Simovic, S., & Prestidge, C. A. (2009a). Chemical stability and phase distribution of all-trans-retinol in nanoparticle-coated emulsions. *International Journal of Pharmaceutics, 376*, 186–194.

Ghouchi-Eskandar, N., Simovic, S., & Prestidge, C. A. (2012). Solid-state nanoparticle coated emulsions for encapsulation and improving the chemical stability of all-trans-retinol. *International Journal of Pharmaceutics, 423*, 384–391.

Gonçalves, A., Estevinho, B. N., & Rocha, F. (2016). Microencapsulation of vitamin A: A review. *Trends in Food Science & Technology, 51*, 76–87.

Hu, M., McClements, D. J., & Decker, E. A. (2003). Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 51*(5), 1435–1439.

Jenning, V., & Gohla, S. H. (2001). Encapsulation of retinoids in solid lipid nanoparticles (SLN). *Journal of Microencapsulation, 18*(2), 149–158.

Kupongsak, S., & Sathitvorapojjana, S. (2017). Properties and storage stability of O/W emulsion replaced with medium-chain fatty acid oil. *Polish Journal of Food and Nutrition Sciences, 67*(2), 107–116.

Lee, S. C., Yuk, H. G., Lee, D. H., Lee, K. E., Hwang, Y. I., & Ludescher, R. D. (2002). Stabilization of retinol through incorporation into liposomes. *Journal of Biochemistry and Molecular Biology, 35*(4), 358–363.

Mao, L., Xu, D., Yang, J., Yuan, F., Gao, Y., & Zhao, J. (2009). Effect of small and large molecule emulsifiers on the characteristics of β-carotene nanoemulsions prepared by high pressure homogenization. *Food Technology and Biotechnology, 47*(3), 336–342.

Marinova, K. G., Alargova, R. G., Denkov, N. D., Velev, O. D., Petsev, D. N., Ivanov, I. B., & Borwankar, R. P. (1996). Charging of oil–water interfaces due to spontaneous adsorption of hydroxyl ions. *Langmuir, 12*, 2045–2051.

McClements, D. (2005). *Food emulsion, principles, practices, and techniques.* Massachusetts, Amherst: CRC Press.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*(8), 1270–1282.

Qi, Z. H., & Shieh, W. J. (2002). Aqueous media for effective delivery of tretinoin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 44*(1), 133–136.

Salvia-Trujillo, L., Qian, C., Martin-Belloso, O., & McClements, D. J. (2013). Modulating β-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. *Food Chemistry, 139*, 878–884.

Sauvant, P., Cansell, M., Sassi, A. H., & Atgié, C. (2012). Vitamin A enrichment: Caution with encapsulation strategies used for food applications. *Food Research International, 46*, 469–479.

Silvestre, M., Chaiyasit, W., Brannan, R. G., McClements, D. J., & Decker, E. A. (2000). Ability of surfactant headgroup size to alter lipid and antioxidant oxidation in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 48*(6), 2057–2061.

Yanaki, T. (2001). Preparation of O/W/O type multiple emulsions and its application to cosmetics. *Studies in Surface Science and Catalysis, 132*, 1009–1014.

Yoshida, K., Sekine, T., Matsuzaki, F., Yanaki, T., & Yamaguchi, M. (1999). Stability of vitamin A in oil-in-water-in-oil-type multiple emulsions. *Journal of the American Oil Chemists' Society, 76*(2), 1–6.

Zhao, Y., Guan, Y., Pan, Y., Nitin, N., & Tikekar, R. V. (2015). Improved oxidative barrier properties of emulsions stabilized by silica–polymer microparticles for enhanced stability of encapsulants. *Food Research International, 74*, 269–274.