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

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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

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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