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Antioxidant activity and bioaccessibility of size-different nanoemulsions for lycopene-enriched tomato extract

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ABSTRACT

Lycopene nanoemulsions were prepared to protect the antioxidant activity and improve the bioaccessibility of lycopene-enriched tomato extract (containing 6% of lycopene) by an emulsification–evaporation method. Lycopene nanoemulsions, with droplet sizes between 100 and 200 nm, exhibited higher anti-radical efficiency and antioxidant activity, than did those smaller than 100 nm. Strong protectability of lycopene in droplets smaller than 100 nm was associated with relatively slower rates of DPPH and ABTS reactions. *In vitro* bioaccessibility values of lycopene-enriched tomato extract, lycopene nanoemulsions with droplets larger than 100 nm (approximately 150 nm on average), and lycopene nanoemulsions with droplets smaller than 100 nm (69 nm on average) were 0.01, 0.53, and 0.77, respectively. Interestingly, nanoemulsions with droplets smaller than 100 nm showed the highest *in vitro* bioaccessibility, which could be interpreted as evidence of nanoemulsification enhancing the *in vitro* bioaccessibility of lycopene.

1. Introduction

Lycopene is the most abundant carotenoid and is the compound responsible for the red colour of tomato products (Shi & Maguer, 2000). Epidemiological studies indicate that high consumption of tomatoes is related to lower risk of cancer and is protective against a number of cardiovascular diseases (Heber & Liu, 2002). However, in spite of its excellent health-supporting function, applications of lycopene in food are limited by the lipid oxidation that occurs during processing and storage.

Lycopene is known to be soluble in oil and sensitive to light and heat; it deteriorates easily during processing and storage, and exhibits poor bioavailability (Nagy, 2009; Shi & Maguer, 2000). Health benefits associated with lycopene consumed in food can be enhanced by preventing its degradation and improving its aqueous solubility through its incorporation into the oil phase within oil-in-water nanoemulsions (Gutierrez et al., 2008; Huang, Yu, & Ru, 2010).

Further food applications of lycopene can be expanded by a proper processing technique; droplet size in the emulsion, in particular, affects its transparency and bioavailability from food. Reduction in droplet size increases the specific surface area and small droplet sizes are desirable for increasing flavor release, transparency, and bioavailability (Aungst, 1993). Additionally, droplet characteristics, such as size and surface area, may affect the oxidation kinetics in oil-in-water emulsions (Lethuaut, Métro, & Genot, 2002; Osborn & Akoh, 2004).

Lycopene bioavailability appears to be dependent on several factors, including lycopene content and composition of the food matrix incorporating lycopene molecules, droplet size during the digestive process, and other factors (During, 2012). Therefore, most studies undertaken so far have been performed under the assumption that an increase in aqueous solubility (and intestinal cell uptake) plays an important role in enhancing the bioavailability of bioactives (Acosta, 2009).

As described previously, modification of the physical characteristics of the droplets may alter the transparency, release properties, human absorption, and storage stability of lycopene in the emulsions prepared as food products. In our current study, we hypothesized that a smaller droplet size, with proper surface characteristics, may enhance transparency, improve aqueous stability, retard oxidation, and increase the bioaccessibility of lycopene.

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The smaller droplet size and optimal surface properties can be produced by controlling the emulsification conditions, such as the type of homogenizer used, intensity and duration of emulsification, temperature, emulsifier type, and the concentration of emulsifier (McClements & Rao, 2011).

The major objectives of this study were (1) to investigate the effect of modifying the homogenization–evaporation processing conditions (the number of homogenization cycles, lycopene-enriched tomato extract content, and emulsifier content) on the physicochemical characteristics, such as droplet size, emulsification efficiency and storage stability, in the preparation of lycopene nanoemulsions of different droplet sizes and (2) to evaluate the antioxidant activity and bioaccessibility of produced nanoemulsions, focussing on *in vitro* digestion.

2. Materials and methods

2.1. Materials

Lycopene-enriched tomato extract (Lyco-O-Mato 6% lycopene) was donated by Lycored Ltd (Be'er Sheva, Israel). Trolox (6-hydroxy-2, 5, 7, 8-tetramethychroman-2 carboxylic acid) supplied by Hofman-La Roche, Aldrich Chemical Co. (Gillingham, Dorset, UK) was used as an antioxidant standard. Stock trolox solution (1.5 mM) was prepared in 5 mM phosphate buffered saline (PBS, pH 7.4) under gentle ultrasonication to dissolve the crystals. Standard lycopene (>95%), 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and potassium persulfate were supplied by Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, *n*-butanol, methyl chloride, ethyl acetate, and other solvents were supplied by Daejung Chemicals & Metals Co., Ltd. (Shiheung, Korea).

2.2. Preparation of lycopene nanoemulsions

Lycopene nanoemulsions were prepared using the emulsification–evaporation technique. Lycopene-enriched tomato extract was dissolved at a ratio of 5% (w/v) in ethyl acetate while being stirred at 500 rpm for 3 h. The lycopene organic solution was then filtered through a paper membrane. Butylated hydroxytoluene (BHT, 0.01%) was added as an antioxidant to prevent the degradation of lycopene-enriched tomato extract. This organic solution was then poured slowly into the aqueous solution containing 0.5% (w/w) Tween 20 in distilled water while being stirred. The mixture was homogenized, using a shear homogenizer (WiseMix HG-15A, Daihan Scientific Co., Ltd., Wonju, Korea) at 5000 rpm for 5 min, and subsequently passed through the high-pressure homogenizer (Nanomizer, Model HS–0102, Hwasung F&B Ltd., Gwangju, Korea). Finally, ethyl acetate was removed by rotary evaporation (Rotary Evaporator N-1000, Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 30 °C. In order to investigate the effect of homogenization–evaporation processing conditions on physicochemical properties of lycopene nanoemulsions, several processing parameters were studied, including the number of cycles (1, 2, and 3) and homogenization pressures (60, 80, 100, and 140 MPa).

2.3. Lycopene concentration and emulsification efficiency in nanoemulsions

Lycopene concentration was determined by high-performance liquid chromatography (HPLC), according to the previously described procedure (Strati & Oreopoulou, 2011) with minor modifications. HPLC was performed using a L1200 series system (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with a G1322A degasser, G1316A Colcom, and G1311A Quatpump. Lycopene content was estimated, using a C18 column (4.6 × 150 mm, 5 μm, Waters, Milford, MA, USA). A mobile phase, consisting of acetonitrile (A), *n*-butanol (B), and methyl chloride (C) was used with the following gradient elution profile: 69.3% A, 29.7% B, and 1% C initially; changed to 67.2% A, 28.8% B, and 4% C at 10 min following the injection of the sample; 61.6% A, 26.4% B, and 12% C at 20 min; 49% A, 21% B, and 30% C after 40 min, and returned to 69.3% A, 29.7% B, and 1% C after 50 min. All solvents had 0.01% BHT added as an antioxidant, and were filtered and degassed for 30 min prior to use. The flow rate was 1 ml/min, column temperature was 25 °C, and detection was carried out at 472 nm. Linear response was found in a range of 5–50 μg/ml (R^2 = 0.9985).

2.4. Size, polydispersity index and zeta-potential of droplets in the nanoemulsions

Average droplet size and polydispersity index of lycopene nanoemulsions were determined by a commercial zeta-potential and particle size analyzer (DelsaNano, Beckman Coulter, Inc., Fullerton, CA, USA) at 25 °C with a scattering angle of 165°. To measure size and polydispersity, 3 ml of lycopene nanoemulsion were transferred to an optical-grade cell and analyzed for intensity distribution. Droplet sizes were represented as $d_{v,10}$, $d_{v,50}$, and $d_{v,90}$, corresponding to the sizes that are equal to or greater than 10%, 50%, and 90% of the measured droplets, respectively.

Zeta potential of lycopene nanoemulsions was assessed by determining the droplet electrophoretic light scattering (ELS), using the same commercial zeta-potential and particle size analyzer at 25 °C. Zeta-potential of lycopene droplets was scanned on a continuous pH range from 4 to 7. Measurements of both size distribution and zeta-potential were performed at least in triplicate for each sample.

2.5. Transparency analysis

Transparency of lycopene nanoemulsions was determined by measuring the absorbance at 600 nm, using a UV-spectrometer (DU 730, Beckman Coulter, Inc.). The transparency was calculated by the following equation:

$$T = 1/10^{A}$$

where T is the transparency and A is the value of absorbance at 600 nm. According to this equation, a high T value would correspond to a transparent appearance.

2.6. Transmission electron microscopy

Droplet morphology in the lycopene nanoemulsions was observed by transmission electron microscopy (TEM, JEM-2010, Jeol Ltd., Tokyo, Japan). The specimens were prepared on Formvar-filmed grids. The observations were carried out with TEM operating at 100 kV in accelerating voltage.

2.7. Antioxidant activity measurement by DPPH assay

The effect of lycopene nanoemulsions on DPPH radical-scavenging activity was estimated according to the procedure established (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). Unemulsified lycopene-enriched tomato extract and prepared lycopene nanoemulsions were exposed to ambient air at 25 °C. An aliquot of nanoemulsion sample was added to 3.9 ml of DPPH· solution (0.025 g DPPH·/l). Absorbance of the DPPH· mixture at 515 nm was measured, using a UV-spectrometer (DU 730, Beckman Coulter, Inc.) at different time intervals until the reaction reached