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Producing a lycopene nanodispersion: Formulation development and the effects of high pressure homogenization

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ABSTRACT

The aim of this study was to develop formulations to produce lycopene nanodispersions and to investigate the effects of the homogenization pressure on the physicochemical properties of the lycopene nanodispersion. The samples were prepared by using emulsification-evaporation technique. The best formulation was achieved by dispersing an organic phase (0.3% w/v lycopene dissolved in dichloromethane) in an aqueous phase (0.3% w/v Tween 20 dissolved in deionized water) at a ratio of 1:9 by using homogenization process. The increased level of homogenization pressure to 500 bar reduced the particle size and lycopene concentration significantly ($p < 0.05$). Excessive homogenization pressure (700–900 bar) resulted in large particle sizes with high dispersibility. The zeta potential and turbidity of the lycopene nanodispersion were significantly influenced by the homogenization pressure. The results from this study provided useful information for producing small-sized lycopene nanodispersions with a narrow PDI and good stability for application in beverage products.

1. Introduction

Lycopene is a fat-soluble substance that belongs to the carotenoid family of phytochemicals. Lycopene's structure is made of unsaturated straight chain hydrocarbons with the empirical formula $C_{40}H_{56}$. This structure is responsible for lycopene's high hydrophobicity, which makes it insoluble in water and only soluble in oil or certain types of organic solvents. The health benefits of lycopene are attributed primarily to its powerful antioxidant actions (Aydin et al., 2013; Camara, Sanchez-Mata, Camara, & Caceres, 2013). It is more effective in comparison with other types of carotenoids, including beta carotene, in preventing free radicals from damaging the cells in our body (Camara et al., 2013; Campos et al., 2017). Lycopene is believed to play a role in the prevention of heart disease by inhibiting free radical damage to LDL cholesterol (Ayoub, Camargo, & Shahidi, 2016; Karppi, Nurmi, Kurl, Rissanen, & Nyyssonen, 2010). Epidemiological evidence suggests that lycopene can protect individuals from colorectal cancer (Vrieling et al., 2007) and men from prostate cancer (Schwarz et al., 2008).

Although lycopene has many health benefits, its poor water solubility has restricted its application within the food industry and especially in water-based food products. The oral bioavailability (Faisal, Ruane-O'hara, O'Driscoll, & Griffin, 2013) and absorption rate of lycopene in the gastrointestinal tract are also limited because of this poor water solubility. Despite the solubility issue, research into adopting lycopene as a value-added food ingredient by the food industry still remains attractive because it offers various health benefits. Ax, Mayer-Miebach, Link, Schuchmann, and Schubert (2003) reported that the solubility and bioavailability of lycopene can be improved by dissolving it within oil in a water emulsion. Kim, Ha, Choi, and Ko (2014) suggesting incorporation of lycopene nanoemulsion in food products particularly in beverages since it has a highly transparent appearance and improved oxidative stability.

Nanodispersion, i.e., a dispersion containing nano-sized particles, has been reported as an alternative for overcoming the bioavailability problems of bioactive compounds such as functional lipids (Krause & Muller, 2001). Nanodispersion is a system consisting of nano-sized particles surrounded with emulsifiers dispersed in an aqueous phase. The special feature of nanodispersions is the very small particle diameter that increases the surface area, enhancing its absorption ability in the gastrointestinal tract and its oral bioavailability (Faisal

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et al., 2013). Nanodispersion and nanoemulsion both have their own advantages and disadvantages. However, the primary difference between these two systems is the presence of the solvent material. In a nanodispersion, the water-insoluble compound of interest will be dissolved in a volatile solvent that can be easily removed (via evaporation under reduced pressure) at the end of the preparation process. In contrast, a nanoemulsion usually contains the compound of interest dissolved in oil. However, the oil is not removed at the end of the preparation process due to its high boiling point.

Emulsification-evaporation is one of the most preferred techniques for producing a nanodispersion (Anarjan, Mirhosseini, Baharin, & Tan, 2010). This method has been employed to produce water-soluble drugs and functional lipid ingredients such as phytosterol nanodispersions (Leong et al., 2011), carotenoid nanodispersions such as astaxanthin (Anarjan et al., 2010), α-tocopherol (Cheong, Tan, Che Man, & Misran, 2008) and β-carotene (Tan & Nakajima, 2005). In this technique, the organic phase-containing lipid compound that is dissolved in organic solvent and the aqueous phase-containing emulsifier that is dissolved in water are passed through a high shear homogenizer to produce a coarse oil-in-water emulsion. The resulting coarse emulsion is then passed through a high pressure homogenizer to produce a nanoemulsion with a uniform distribution of particles. The conversion of nanoemulsions to nanodispersions occurred when the organic solvent used to dissolve the compound was evaporated off the system. Anarjan, Tan, Nehdi, and Ling (2012) who produced astaxanthin nanodispersion using emulsification-evaporation technique found that the concentration of dichloromethane traces in the prepared sample was < 0.02 ppm. The amount of the dichloromethane traces reported by Anarjan et al. (2012) was below than the permitted level of daily intake for dichloromethane in food processing which is 600 ppm (Witschi & Doelker, 1997).

Recently, a study on the production of lycopene nanoemulsion for applications in beverage (Kim et al., 2014) and also on the improvement of its bioaccessibility (Ha et al., 2015) was reported. However, to the extent of our knowledge, the production of lycopene nanodispersion particularly by emulsification-evaporation technique has not been reported. The novelty of this study is the application of emulsification-evaporation technique to produce lycopene nanodispersion which has features such as small particle size (nano-meter range), low polydispersity index (PDI), high physical stability, improved water solubility and high transparency for food applications particularly in transparent beverages. The goal of this research is to find the best formulation for producing lycopene nanodispersions with the desirable characteristics while investigating the effects of high pressure homogenization on the properties of the prepared lycopene nanodispersion.

2. Materials and methods

2.1. Materials

Lycopene (50%) was purchased from Shaanxi Jinjiankang Biological Technology Co., Ltd. (Xi'an, China). Tween (T20), HPLC-grade dichloromethane, tetrahydrofuran, methanol, acetone, hexane, ethanol, ethyl acetate and acetonitrile were purchased from Fisher Scientific (Leicestershire, UK).

2.2. Lycopene powder solubility in organic solvent

Lycopene powder (0.3% w/v) was dispersed into different types and mixtures of organic solvents with ratio of 1:1 and 1:1:1 (dichloromethane, hexane, acetone, ethanol, methanol, ethyl acetate, acetonitrile, dichloromethane:acetone, dicholoromethane:hexane, hexane:acetone, hexane:acetone:ethanol and hexane:acetone:dichloromethane) by magnetic stirring at room temperature for 5 h. The solubility of the lycopene powder was examined by observing the transparency of the solution, and the color and presence of undissolved lycopene powder. The organic solvent that could dissolve the lycopene powder was selected to prepare the lycopene nanodispersion which was then analyzed for particle size and PDI.

2.3. Preparation of lycopene nanodispersion

2.3.1. Coarse emulsion preparation

An organic phase was prepared by dissolving lycopene powder (0.3% w/v) in dichloromethane. The mixture was stirred at room temperature for 5 h. The aqueous phase was prepared by dissolving an emulsifier (T20) (0.3% w/v) in deionized water for 1 min with a high shear mixer (Silverson L4R, Buckinghamshire, UK). The organic phase and aqueous phase were then mixed using a high shear mixer (Model Silverson L4R, Buckinghamshire, UK) for 5 min at 5000 rpm to form coarse O/W emulsions. The ratio of organic to aqueous phase was 1:9.

2.3.2. Emulsification and evaporation

Fine emulsions were formed by passing the resulting coarse emulsions through a high-pressure homogenizer (Panda Plus 2000; GEA Niro Soavi, Parma, Italy) for 1 cycle at different pressures. The lycopene nanodispersions were obtained by removing the organic solvent in the resulting fine emulsions using a rotary evaporator (Eyela NE-1001, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) under reduced pressure of 25 kPa at 40 °C and with rotation speed of 100 rpm. The principle of sample preparation for the lycopene nanodispersion is shown schematically in Fig. 1.

2.4. Characterization of lycopene nanodispersion

2.4.1. Particle size and polydispersity index determination

Mean particle size and PDI of a lycopene nanodispersion were determined by using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The measurement was performed on the basis of backscatter angle of 173°. The mean particle diameters were reported as “Z-average” diameters (the scattering intensity-weighted mean diameter) in nm. An aliquot of freshly prepared nanodispersion was placed in a 1 cm path length cuvette and inserted into the cuvette holder. The sample was equilibrated at 25 °C for 120 s before measuring the particle size and PDI at 25 °C.

2.4.2. Zeta potential determination

The zeta potential of lycopene nanodispersion was determined using Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). Freshly prepared sample (1 mL) was injected into a disposable folded capillary cell (DTS 1060) and placed in the cell holder before being equilibrated at 25 °C for 120 s.

2.4.3. Lycopene concentration determination

The extraction of lycopene from the freshly prepared sample was performed following the procedure described by Anarjan and Tan (2013), with minor modifications. Lycopene nanodispersion (1 mL) was mixed with 2 mL of hexane:acetone (2:1) and vortexed at 1000 rpm for 5 min. Then, the sample was centrifuged at 800 g for 10 min. The hexane upper layer containing lycopene was collected, and the extraction method was repeated once again. The lycopene concentration in the hexane layer was then subjected to HPLC analysis. One millilitre of the collected hexane layer was filtered through a syringe filter (0.45 μm), and 20 μL of the filtrate was then injected into an HPLC system (Waters, Milford, MA, USA) equipped with a Diode Array Detector and a Nova-Pak C18 (3.9 × 300 mm) Waters HPLC column. An isocratic mobile phase (15% v/v tetrahydrofuran, 30% v/v acetonitrile and 55% v/v methanol) was used and the measurement was performed at 472 nm. All steps were performed under subdued light. Theoretically, after homogenization and evaporation, the final concentration of lycopene in the emulsion should be 0.06 g lycopene in 360 mL water, which is equivalent to 60 mg/360 mL, or approximately 16.7 mg/100 mL.