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Formulation of oil-in-water β-carotene microemulsions: Effect of oil type and fatty acid chain length

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ABSTRACT

The impact of oil type and fatty acid chain length on the development of food-grade microemulsions for the entrapment of β-carotene was investigated. The microemulsion region of a ternary phase diagram containing short chain monoglycerides was larger than for di- and triglycerides when Tween 80 was used as surfactant. The cytotoxicity of microemulsions composed of a 30% monoglyceride oil, 20% Tween 80 and 50% aqueous buffer were evaluated using an *in vitro* cell culture model (human epithelial colorectal adenocarcinoma, Caco-2). The cytotoxicity test showed that the viability of Caco-2 cells against β-carotene microemulsions at concentrations of 0.03125% (v/v) was higher than 90%. This study suggests that short chain monoglycerides could be used with Tween 80 to prepare transparent β-carotene-encapsulated O/W microemulsions in the particle size range of 12–100 nm.

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1. Introduction

Fortification of food and beverages with oil-soluble bioactive components is a major initiative within the food industry to improve population health (McClements, Decker, Park, & Weiss, 2009). Carotenoids are a class of lipophilic pigment molecules which are widespread among different fruits and vegetables as well as some animal products (Yonekura & Nagao, 2007). β-Carotene, one of the most commonly available carotenoids in the human diet, is a well-known active phytochemical with health promoting properties against lung cancer (Albanes et al., 1996), heart disease (Omenn et al., 1996) and colorectal adenomas (Jung et al., 2013).

Although the development of functional foods and beverages has grown rapidly in recent decades, the utilisation of β-carotene as a functional ingredient is limited due to its poor water solubility, chemical instability and low bioavailability (Liang, Shoemaker, Yang, Zhong, & Huang, 2013). To overcome these limitations, a number of different β-carotene delivery systems have been investigated, including oil-in-water (O/W) nanoemulsions (Liang et al., 2013), nanodispersions (Yin, Chu, Kobayashi, & Nakajima, 2009), nanostructured lipid particles (Hejri, Khosravi, Gharanjig, & Hejazi, 2013) and liposomes (Moraes et al., 2013). These delivery systems for β-carotene may remain stable for a considerable period of time; however, they are not thermodynamically stable, thus eventually undergoing destabilization (Flanagan & Singh, 2006). Moreover, the problem of fortification of food and beverages with β-carotene by those systems alters the optical properties of products, increasing their turbidity and consequently changing the appearance which is particularly detrimental for the production of transparent food and beverages. Therefore, the potential of alternative delivery systems such as microemulsions should be explored to solve these problems.

Microemulsions are defined as thermodynamically stable, isotropic and transparent emulsions of oil and water stabilized by an interfacial film of a suitable surfactant, and with droplet size smaller than 100 nm (Flanagan & Singh, 2006). The characteristics of microemulsions that find applications in food industry, as well as other industrial applications, are (i) transparency, which is important for clear beverages; (ii) small droplet size, which plays a role in flavour release; (iii) increased solubilisation and bioavailability of bioactives; (iv) protection of solubilised components from degradative reactions; and (v) high stability which is an

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added benefit during processing and storage of beverages (Gaonkar & Bagwe, 2002). In spite of the vast potential for these promising applications, the application of microemulsions in the food industry is limited by the type of lipids and formulation methods.

The types of lipids used in oral lipid-based formulations can be classified by chemical structure, composition and properties (polarity) as long chain triglycerides (LCTs; e.g., soybean oil), medium chain triglycerides (MCTs; e.g., fractionated coconut oil, Captex 200, Captex® 355), propylene glycol esters of fatty acids (mono-/diglycerides; e.g., Capmul® PG-8, Capmul® MCM, Captex 200), fatty acids (e.g., oleic acid, palmitic acid, linoleic acid) and lipid mixtures (e.g., Gelucire® 33/01, Phosal® 53MCT) (Cannon & Long, 2008). The efficacy of these lipids as carriers of lipophilic compounds can vary according to the physicochemical properties of the delivery systems, and by the preparation and processing methods (Prajapati, Dalrymple, & Serajuddin, 2012). The lipids commonly used for making food-grade microemulsions are mostly unsaturated long-chain triglycerides (Flanagan, Kortegaard, Neil Pinder, Rades, & Singh, 2006); however, these can be too bulky to penetrate the interfacial film to assist in the formation of small droplets (Gaonkar & Bagwe, 2002). To minimise this problem, a co-surfactant is often employed to further lower the interfacial tension (Garti, Yaghmur, Leser, Clement, & Watzke, 2001). Using co-surfactants (e.g., ethanol) may not be suitable for use in foods because short- and medium-chain alcohols can cause toxicity and irritation (Flanagan & Singh, 2006).

The effect of oil type on the encapsulation of β-carotene in microemulsions was examined. Pseudo-ternary phase diagrams of microemulsions prepared from long chain triglycerides (soybean oil) and mono, di- and triglycerides of medium chain fatty acids were constructed using food-grade ethoxylated sorbitan esters (Tweens). The cytotoxicity of β-carotene microemulsion was investigated using an *in vitro* cell culture model.

2. Materials and methods

2.1. Materials

Soybean oil, β-carotene crystals (purity 99.8%), Tween 20 (polyoxyethylene sorbitan monolaurate), Tween 40 (polyoxyethylene sorbitan monopalmitate) and Tween 80 (polyoxyethylene sorbitan monooleate) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Glycerol monocaprylocaprate (Capmul MCM), propylene glycol dicaprylate/dicaprate (Captex 200) and caprylic/capric triglyceride (Captex 355) were kindly donated by Abitec Corporation (Janesville, WI, USA). Ethanol (95%) and acetone were purchased from Biolab (Scoresby, Victoria, Australia). Sodium phosphates ($Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4.H_2O$) and n-hexane were obtained from J.T. Baker (Phillipsburg, NJ, USA). All chemicals and solvents used in this experiment were of analytical or HPLC grade.

2.2. Solubility of β-carotene

Solubility of β-carotene in different oils (soybean oil and mono-, di- and triglyceride of medium chain fatty acids) and surfactants (Tween 20, 40 and 80) was determined in triplicate by dissolving an excess amount of β-carotene (~4 mg) in 1 mL of oil or surfactants. Samples were then blanketed with nitrogen, sonicated (Elmasonic S40, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany, 37 kHz, 560 W) for 30 min, placed in a thermostatic orbital shaker (Model OM 11, Ratek Instruments Ltd., Boronia, Victoria, Australia) and continuously shaken at 300 rpm for 48 h at 30 °C. The equilibrated samples were then transferred to a polyallomer centrifuge bottle (Beckman, Fullerton, CA, USA) and centrifuged at 60,800×*g* for 15 min to remove undissolved β-carotene, and the clear supernatant liquid fraction was decanted. The supernatant was filtered through 0.22 μm hydrophobic polytetrafluoroethylene syringe filters (Millipore, Carrigtwohill, Co. Cork, Ireland) to remove any remaining insoluble β-carotene. After diluting in methanol, the β-carotene concentration in the filtrate was quantified using reversed phase C_{18} HPLC assay as described below.

2.3. Microemulsions preparation and phase diagram construction

Four pseudo-ternary phase diagrams containing oil-surfactant-water were constructed to define the microemulsion region. Samples were prepared by mixing appropriate amounts of long chain triglycerides (soybean oil) or medium chain triglycerides (Capmul MCM, Captex 200 and Captex 355), phosphate buffer (0.01 M, pH 6.8) and surfactant (Tween 20, 40 or 80) in vials at room temperature and mixed using a vortex mixer. The pseudo-ternary phase diagrams were constructed by preparing samples of 100 different compositions to define the phase boundaries in each phase diagram using SigmaPlot software (version 12.3, Systat Software Inc., Chicago, IL, USA). Each sample was further allowed to equilibrate at room temperature for at least 24 h before evaluation and re-examined after one week. Depending upon the components used, four different phases were observed in the phase diagrams: (i) a clear liquid region which included a clear or transparent water-in-oil (W/O) microemulsion; (ii) a clear liquid region which included a clear or transparent O/W microemulsion; (iii) a viscous gel, and (iv) a phase-separated mixture where the lipid separated from the aqueous phase to form a separate layer. All selected microemulsions were stored at room temperature and the stability of each sample was assessed by visual inspection in terms of clarity over time.

2.4. Determination of microemulsion type using dye and electrical conductivity tests

The type of microemulsions (O/W and W/O) was identified by a staining method. The water-soluble dye methylene blue and the oil-soluble dye Sudan III were added in equal amounts to blank microemulsions (microemulsions without β-carotene) to evaluate the diffusion rate of these two dyes. A faster diffusion for methylene blue indicates an O/W microemulsion, and faster diffusion for the red-coloured Sudan III indicates a W/O microemulsion. Conductivity measurements were carried out for the microemulsion samples using an electrical conductivity meter (CyberScan CON 11, Eutech Instruments, Singapore). The electrode was dipped in the microemulsion samples until equilibrium was reached and the reading became stable. The reproducibility of test results was checked for the samples and no significant differences were observed. The samples were thermostated at 25 °C.

2.5. Extraction of β-carotene from microemulsions

The extraction of β-carotene from microemulsions was carried out according to the method of Wright, Pietrangelo, and MacNaughton (2008). Briefly, samples (1 mL) were subjected to solvent extraction by adding ethanol (0.5 mL), acetone (3 mL), and deionized water (1 mL) with 5 s of vortexing after the addition of each liquid. After adding 2 mL of hexane the vials were shaken by inverting ten times and the organic layer was removed after 5 min using a glass transfer pipet. This extraction was repeated three times for each sample. Hexane (6 mL in total for each sample) was pooled and the organic phase was dried under nitrogen at 37 °C to the point of dryness. HPLC-grade methanol (1 mL) was added and vortexed for 5 s.

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