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Optimization of formulation and influence of environmental stresses on stability of lycopene-microemulsion

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ABSTRACT

The stability of two-layer lycopene-microemulsions and the degradation of lycopene in microemulsions subjected to thermal processing and under environmental stress were investigated. The formulation of microemulsions made with different ratios of whey protein isolate (WPI), high-methylester-pectin (HMP), and an oil phase containing lycopene was optimized. The oil volume fractions, concentrations of WPI and HMP, and their interactions significantly influenced the physical stability of lycopene-rich microemulsions. The two-layer microemulsion consisted of WPC and HMP was much more stable under environmental stress compared to the WPC one-layer microemulsions. The one-layer lycopene-microemulsions destabilized at low pH, but the two-layer lycopene-microemulsion became unstable when the pH was in the neutral range of 6.12 to 7.01. The stability of microemulsions declined with increasing NaCl concentrations. About 36.8 g/100 g, 23.2 g/100 g, and 11.1 g/100 g of the total lycopenes were lost in the oil-phase, the one-layer lycopene-microemulsions, and the two-layer lycopene-microemulsions after thermal treatments, respectively. The optimized microemulsions contained 0.2 g/100 g (w/w) whey protein concentrate (WPC), 0.5 g/100 g HMP (w/w), and 5 mL/100 mL oil phase fraction had the highest physical stability. The optimized lycopene-microemulsion was more stable to thermal treatment and changes of pH, but become sensitive to NaCl treatment.

1. Introduction

Lycopene is one of many important carotenoids responsible for the fragrance of vegetables and fruits. It has important biologic activities associates with antioxidant properties and with stimulation of cell-to-cell communication (Kun, Lule, & Xiao-Lin, 2006). In terms of health issues, the intake of lycopene has been associated with a reduction in the incidence of some cancers, coronary heart disease, and aging (Bramley, 2000; Kun et al., 2006; Rao & Agarwal, 2000; Wilcox, Catignani, & Lazarus, 2003). The incorporation of lycopene into food and beverages has been limited due its poor solubility in water and low stability when exposed to environmental stresses such as heat, light and oxygen (Lee & Chen, 2002; Shi, Dai, Kakuda, Mittal, & Xue, 2008). Numerous studies have suggested that the bioavailability of lycopene could be enhanced when mixed with dietary fats. This would imply that the amount and type of dietary fat in the food product will increase the incorporation of free lycopene and enhance its bioavailability (Deming, Boileau, Lee, & Erdman, 2000; Hoppe, Krämer, van den Berg, Steenge, & van Vliet, 2003; Paiva & Russell, 1999). The molecular configuration (*cis*- and *trans*-) of the lycopene isomers can also affect its bioavailability (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999). Therefore, the major challenges that must be resolved before lycopene can be expediently incorporated into food products are their low water solubility, physical and chemical instability, and poor bioavailability, and thus to extend lycopene applications from pharmaceutical into beverage and food productions.

The emulsion system has shown to improve the solubility, stability and bioavailability of lipophilic compounds (McClements, 2012; McClements, Decker, & Weiss, 2007). The oil-in-water emulsion can be used to encapsulate lipophilic lycopene molecule, and also can be conveniently incorporated into water-based

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food system such as beverages, and food products. The oil-formed lycopene in the emulsion would enhance their bioavailability after consumption. Moreover, the emulsifier layer surrounding the oil droplets that containing lipophilic lycopene can be designed to protect the lycopene against oxidation and degradation by reducing them exposure to oxygen, interact with metal ions such as Fe^{2+}, heat, and light (Mao et al., 2009; McClements & Decker, 2000). A number of researchers have prepared emulsions containing carotenoids. Ax, Miebach, Link, Schuchmann, and Schubert (2003) used a high pressure homogenizer to prepare lycopene emulsions, and evaluated the thermal stability and oxidative stability of the lycopene-emulsion system. They found the total lycopene content of the oil-in-water emulsions decreased during thermal treatment with and without exposure to oxygen. However, the addition of tocopherol containing sunflower oil to the emulsion system reduced lycopene degradation during thermal treatment. Tan and Nakajima (2005) used the emulsification-evaporation technique to prepare β-carotene nanodispersions. The characterization of these nanodispersions showed that the degradation of β-carotene was dependent on the mean diameter of the particles and the emulsion was chemically unstable during storage at 4 °C for 12 weeks. Qian, Decker, Xiao & McClements (2012) studied the physical and chemical stability of β-carotene-enriched nanoemulsions under different pH, ionic strength, temperature and emulsifier type. They reported that encapsulated β-carotene had a tendency to chemically degrade which led to colour fading over time during storage. The rate of colour fading increased with increasing storage temperature, had the fastest rate at the lowest pH value (pH = 3), and was largely independent of salt concentration (0–500 mM NaCl). Several studies reported that microencapsulation offered greater protection to lycopene by atomization and spry-drying methods using modified starch gum arabic and sucrose, gum arabic and maltodextrin, and gelatin and sucrose as encapsulants materials (Glaucia, Carmen, & Carlos, 2012; Matioli & Rodriguez-Amaya, 2002; Nunes & Mercadante, 2007). However, the preparation of lycopene-emulsions were lycopene dissolved in chemical agent (hexane or acetone), then the lycopene sample preheated to dissolve in acetone (Shu, Yu, Zhao, & Liu, 2006). Some lycopene-microcapsules were prepared by dissolving lycopene crystals in dichloromethane (Nunes & Mercadante, 2007). Moreover, consumers have been aware that chemicals incorporated in food products have negative impact, and not be acceptable in their diet.

There is a growing trend within the food industry to use the natural, "label friendly" emulsifiers and to replace the synthetic ones. In oil-in-water emulsions, proteins are usually used as one-layer emulsifying agents to form a strong adsorbed layer at the interface to cover the oil droplets, and to stabilize them in coalescence (Amir, Amanda, Wright, & Milena, 2010). Whey proteins are widely used in food applications due to their low cost, high nutritional value, and excellent emulsifying properties (Dickinson, 2003; Gancz, Alexander, & Corredig, 2006; Neirynck et al., 2007). Along with proteins, hydrocolloids such as gum arabic, guar gum, carrageenan, and pectin can be added to improve emulsion stability (Mirhosseini, Tan, Hamid, & Yosuf, 2008). However, the formulation needs to be carefully controlled, because the interactions between hydrocolloids and the adsorbed proteins may initiate aggregation or creaming behaviours such as bridging and depletion flocculation (Gancz et al., 2006). The strength of these interactions is dependent on environmental conditions (e.g., pH and ionic strength), the molecular weight, and the charge of the polysaccharide involved (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2008; Ogawa, Decker, & McClements, 2003; Surh, Gu, Decker, & McClements, 2005). Sunflower oil and medium-chain triglycerides (MCT) have been used mostly for emulsion or encapsulation of carotenoids in the literature (Dimakou & Oreopoulou, 2013; Fernández-García, Rincón, & Pérez-Gálvez, 2008; Lei, Liu, Yuan, & Gao, 2014; Xu et al., 2013). Corn oil has higher amount of unsaturated fatty acids that reduces the risk of lycopene oxidation (Popescu & Soceanu, 2010). Boon et al. (2008) reported that corn oil exhibit higher protective capacity for lycopene against oxidative degradation. Moreover, corn oil is much cheaper and easily accepted by beverage and food industry compare to other oil carriers such as sunflower oil and MCT. Because of product and processing cost are also important concerns for food industry to develop new products or apply new technologies. However, there is no one composition of emulsion that can be ideal for use in every type of food product. Therefore, the selection of the most appropriate emulsion for a particular food product should be based on the type and concentration of emulsifier, oil carrier, and other ingredients in food, processing methods and conditions, and the environmental conditions that it experiences during its manufacture, storage and utilization.

The formulation of lycopene-emulsion and processing conditions. e.g., the volume and proportion of oil-phase, the interactions between surfactants in double layers, the stability of both microemulsion and lycopene, are very limited. Therefore, it is important to determine the affecting factors and their interactions for designing, optimizing and formulating of lycopene-rich microemulsions in an effective and economic way to expend lycopene applications in functional foods and beverages. Response surface methodology (RSM) is a powerful statistical and mathematical tool, and it has a major advantage over the one-factor-at-a-time approach in that it allows the evaluation of the effect of multiple variables and their interaction on the output variable with a reduced number of trails. RSM is a very established and developed method that commonly used for optimizing processing conditions. The aim of present study was to develop lycopene-microemulsions. A three-factor central composite design (CCD) was used to study the effects of three independent variables, namely whey protein isolate (WPI) and high-methylester-pectin (HMP) with various concentrations, oil-phase volumes, and their interactions on the stability of lycopene-rich double-layers microemulsion. Moreover, the stability of the microencapsulated lycopene, in the optimized formulation of microemulsions, was also investigated under varying environmental conditions of pH, ionic strength, and temperature. The study will extensively determine and characterize the microemulsion process and parameters as a useful step in the development of lycopene-rich microemulsion for applications in water-based food systems in commercial production.

2. Materials and methods

2.1. Materials

Whey protein concentrate (protein content >80%) was kindly provided by Davisco Foods International Inc. (MN, USA). The high-methylester-pectin (HMP), citric acid, sodium benzoate, potassium sorbate and sodium sulphate were purchased from Sigma Scientific (Sigma Canada). Corn oil was purchased at a local supermarket. Lycopene extracts were used for this study. The lycopene extracts were prepared in our lab by supercritical CO_2 extraction of tomato waste (Shi et al., 2009).

2.2. Preparation of lycopene-rich emulsion

The oil-in-water lycopene-rich microemulsions were prepared using corn oil (containing 100 μg/mL lycopene extract) as the oil phase. Whey protein isolate (WPI) (0.2 g/100 g–0.5 g/100 g, w/v), and high-methylester-pectin (HMP) – (0.2 g/100 g–0.5 g/100 g, w/v) along with sodium benzoate (0.1 g/100 g, w/w), potassium sorbate (0.1 g/100 g w/w), and deionized water, formed the