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Quantification and visualization of α-tocopherol in oil-in-water emulsion based delivery systems by Raman microspectroscopy

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ABSTRACT

Emulsion is a commonly investigated bioactive loaded delivery system. The bioactive content and its location in oil phase primarily determine the quality and chemical stability of emulsion. In this study, Raman microspectroscopy was used to quantify α-tocopherol and to visualize its distribution in oil-in-water emulsion stabilized by whey protein isolates. Results suggested that α-tocopherol contents (25–300 g/kg) in corn oil with the integrating Raman intensity at 481.9 and 588.6 cm^{-1} showed determination coefficient (R^2) of 0.98 and 0.99, and limit of detection of 5.1 and 21.2 g/kg, respectively. For detecting α-tocopherol in emulsions, the relative standard deviation values from Raman method using intensities at 481.9 cm^{-1} and 588.6 cm^{-1} were in the ranges of 4%–16% and 2%–6%, respectively. The developed Raman method provided correlative results with those of HPLC method (R^2 = 0.99). Moreover, Raman chemical imaging depicted the non-homogeneous TOC distribution within oil droplets, where TOC had the trend of migrating to the interface of oil and water. This study provided a novel approach for functional emulsion analysis, which may serve the basis for designing stable and controllable release of emulsion systems in future.

1. Introduction

In the modern society, demands for high quality and safety foods have been ever increasing, and the food industry not only requires processing techniques such as cooling (Desmond, Kenny, Ward, & Sun, 2000; Hu & Sun, 2000; McDonald & Sun, 2001; McDonald, Sun, & Kenny, 2001; Sun & Eames, 1996), freezing (Cheng, Sun, & Pu, 2016; Cheng, Sun, Pu, & Wei, 2018; Cheng, Sun, Zhu, & Zhang, 2017; Kiani, Sun, Delgado, & Zhang, 2012; Ma et al., 2015; Pu, Sun, Ma, & Cheng, 2015; Qu, Sun, Cheng, & Pu, 2017; Xie, Sun, Xu, & Zhu, 2015; Xie, Sun, Zhu, & Pu, 2016) and drying (Ma, Ji; Sun, Qu, & Pu, 2017; Pu & Sun, 2017; Pu & Sun, 2016; Qu, Sun, Cheng, & Pu, 2017; Sun & Woods, 1993; Sun & Woods, 1994; Sun & Woods, 1994; Sun & Woods, 1994; Yang, Sun, & Cheng, 2017) to enhance food quality and safety, but also needs detection techniques and methods for quality and safety assurance. In addition, consumers also tend to demand for more functional food products that could provide additional health benefits and lessen the burden of disease beyond their natural nutritional attributes (Celli, Ghanem, & Brooks, 2015). Bioactive ingredients, taking the capacity to scavenge free radicals and reduce oxidative stress, have drawn much attention over the past few decades. Vitamin E is a fat-soluble essential nutrient derived from various crops. It has a fundamental role in protecting cell membranes against damage due to its powerful biological antioxidant capacity of terminating chain reactions and chemically preventing lipid oxidation (Goon, Azman, Ghani, Hamid, & Ngah, 2017). Therefore, vitamin E can contribute to preventing risk of some chronic diseases, such as cardiovascular diseases and cancer. α-, β-, γ- and δ-Tocopherols are four isomers of vitamin E. The different structures and physicochemical properties of these four isomers determine their activity and bioavailability. Among these vitamin E series, α-tocopherol (TOC) is the most abundant and most biologically active form of vitamin E compound (Yang & McClements, 2013). Due to its promising therapeutic potential and safety, TOC is increasingly used in fortifying foods and beverages, and drugs (Velikov & Pelan, 2008). However, there are several challenges for developing innovative products with bioactive ingredients, as most of the bioactives have poor

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water-solubility, chemical instability, and poor bioavailability. For example, the highly lipophilic property of TOC lowers its ability in direct disperse into aqueous media, leading to easy degradation when it is exposed to environmental factors, such as oxygen and light, as well as conditions encountered inevitably in the gastrointestinal tract (Liang, Line, Remondetto, & Subirade, 2010).

In order to protect the bioactive ingredients from degradation during storage and improve their bioavailability and pharmacodynamics, a wide variety of effective delivery systems including emulsions, solid lipid nanoparticles, and polymer particles, have recently been developed in the food and nutraceutical industries. In the case of emulsion delivery systems, the stability and release properties of bioactive ingredients mainly depend on their location and partitioning characteristics in oil and water phases. Therefore, it is essential to develop effective detection approaches for monitoring the content and location of bioactive ingredients in emulsion systems, which are crucial for designing physically stable emulsion systems for carrying bioactive ingredients.

Conventional methods for detecting these compounds mainly use ultraviolet-visible (UV-Vis) spectrophotometry or high performance liquid chromatography (HPLC). Guan, Wu, and Zhong (2016) employed a two-step method to extract β-carotene from nano-emulsions improved by eugenol and use a UV-Vis spectrophotometer at 450 nm to measure the concentration of β-carotene in the emulsion. Wang et al. (2016) quantified TOC in oil-in-water (O/W) emulsions stabilized by whey protein isolate using HPLC. Undoubtedly, neither the spectrophotometry nor HPLC method can be employed for on-line and in situ monitoring. Tedious sample preparation processes including centrifugation, separation and extraction are required in order to measure bioactive contents in oil or water phase using these conventional methods. The extraction process also expends many organic chemicals, which are generally environment unfriendly. More importantly, conventional methods can only detect the contents of bioactive ingredients, but cannot obtain their distribution characteristics in oil drops, and information on their spatial distribution is valuable for achieving stable and control-released functional emulsion systems. Therefore, the development of emerging rapid and nondestructive approaches for simultaneous detection of food component contents and their spatial distributions is of great importance, and many studies have been conducted (Cheng & Sun, 2015; Cheng, & Sun, 2017; Cheng, Sun, & Cheng, 2016; Cheng et al., 2016; Cheng, Sun, Pu, Wang, & Chen, 2015; Cheng, Sun, Pu, & Zhu, 2015; Dai, Cheng, Sun, Zhu, & Pu, 2016; ElMasry, Sun, & Allen, 2013; Li, Sun, Pu, & Jayas, 2017; Ma, Sun, & Pu, 2016; Pu, Kamruzzaman, & Sun, 2015; Pu, Liu, Wang, & Sun, 2016; Pu, Xie, Sun, Kamruzzaman, & Ma, 2015; Xiong, Sun, Pu, Xie, Han, & Luo, 2015; Xu, Riccioli, & Sun, 2016).

In recent years, for food quality evaluation, the potential of using emerging imaging, spectroscopic and spectral imaging techniques such as computer vision (Du & Sun, 2005; Jackman, Sun, & Allen, 2011; Sun & Brosnan, 2003; Xu, Sun, & 2017; Xu, Riccioli, & Sun, 2017), infrared spectroscopy (Downey, 1998; Moscetti et al., 2017; Woodcock, Fagan, O'Donnell, & Downey, 2008), Raman spectroscopy (Scheier, Bauer, & Schmidt, 2014), terahertz time-domain spectroscopy (Wang, Sun, & Pu, 2017), hyperspectral imaging (Cheng & Sun, 2015), multispectral imaging (Pu, Kamruzzaman, & Sun, 2015) and Raman chemical imaging (Yakes et al., 2017) has been extensively explored. Especially, Raman microspectroscopy (RMS) as a non-invasive and label-free imaging technique enables rapid analysis of chemical components and their distributions, thus has drawn much attention for food products authentication in recent years (Clemente, Aznar, & Nerin, 2016; Fowler et al., 2015; Pan, Pu, & Sun, 2017; Qin, Chao, Kim, & Cho, 2015).

Raman microspectroscopy is a technique integrating Raman spectroscopy with digital imaging into one instrument to simultaneously obtain both the spectral and spatial information. RMS has several advantages compared with other spectroscopy and imaging techniques. Firstly, Raman spectroscopy gives molecular vibration information based on the scattering effect, which is less affected by water interference. Therefore, RMS technique can conveniently measure samples in various states such as dried or hydrated, liquid- or solid-state with few sample preparation procedures, which is critical for in situ and on-line authentication of food products. Besides, the resultant Raman spectrum generally shows more well-resolved peaks, which can provide particular information regarding the structure, symmetry, electronic environment and bonding of the molecule for quantitative analysis. All of these advantages make RMS suitable for simultaneously detecting chemicals concentrations and their distributions in food products. With the developments in instruments and nanomaterial science, in the past few years, RMS technique has been used in many areas, such as evaluating adulterants and additives in milk powder and wheat flour (Qin et al., 2015, 2017), detecting pesticide residue in agri-food products (Yang et al., 2017), monitoring accumulation of lycopene during tomato ripening (Qin, Chao, & Kim, 2011), and examining component changes in plant and microorganism cells (Clemente et al., 2016; Pan et al., 2017).

On the other hand, RMS has been applied to study complex multiphasic systems. Liu et al. (2016) used confocal Raman microscopy to map the phase of starch-gelatin blended film, in which the dispersion of starch in a continuous gelatin matrix could be visualized. Roeffaers et al. (2011) and Smith, Holroyd, Reid, and Gordon (2016) investigated the distribution of main components and additives in two practical emulsion-based foods (i.e., cheese and mayonnaise), and showed that images of the distribution of lipids, protein, trisodium citrate, and water could be obtained. In addition, quantification of β-carotene partitioning in O/W emulsions was recently validated using confocal Raman microscopy by Mohamad, Buckow, Augustin, and Mcnaughton (2017).

Despite the above studies, study on bioactive ingredients distribution in emulsion systems is lacking. Therefore, in the present study, in order to gain further comprehensive understanding of the location and partition characteristics of the bioactive, TOC loaded O/W emulsions stabilized by whey protein isolate were constructed, and based on the RMS method, the concentration of TOC in the O/W emulsion was correlated with the acquired TOC characteristic Raman spectra, non-homogeneous location of TOC in the oil phase was examined, and the interaction between TOC and whey protein isolate in the water phase was demonstrated. It is hoped that this study should provide a basis for designing stable emulsions with targeted delivery and controlled release behaviors to specific sites.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) with a protein content of > 91% was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA). α-Tocopherol was obtained from Aladdin Reagent (Shanghai) Co., Ltd. (Shanghai, China). Corn oil was bought from a local supermarket (Guangzhou, China) and purified with Florisil (0.15–0.3 mm sieve sizes) (Sigma-Aldrich Co., St. Louis, USA) to remove surface-active impurities. Methanol and hexane with HPLC grade were used in this study. All other chemicals used were of analytical grade.

2.2. Formulation of α-tocopherol loaded emulsions

The aqueous phase was prepared by dissolving 10 g WPI in 100 mL distilled water containing 0.02 g NaN_3 to inhibit microorganism contamination, and the pH was adjusted to 7.0 using 0.1 mol/L NaOH or 0.1 mol/L HCl as required. The solution was stirred at room temperature for 2 h to allow enough hydration before emulsion preparation. The oil phase was prepared by dissolving TOC in corn oil at a ratio of 0, 25, 50, 100, 150, 20 and 300 g/kg. The TOC loaded O/W emulsions were formulated at 25 °C by homogenizing 20 mL oil phase and 30 mL aqueous phase together using a high speed dispersion homogenizer (FJ-