Food Chemistry 239 (2018) 75–85

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Enhancing physicochemical properties of emulsions by heteroaggregation of oppositely charged lactoferrin coated lutein droplets and whey protein isolate coated DHA droplets

Xin Li [a], Xu Wang [a], Duoxia Xu [a,*], Yanping Cao [a,*], Shaojia Wang [a], Bei Wang [a], Baoguo Sun [a], Fang Yuan [b], Yanxiang Gao [b]

[a] *Beijing Advanced Innovation Center for Food Nutrition and Human Health (BTBU), School of Food & Chemical Engineering, Beijing Engineering and Technology Research Center of Food Additives, Beijing Higher Institution Engineering Research Center of Food Additives and Ingredients, Beijing Key Laboratory of Flavor Chemistry, Beijing Laboratory for Food Quality and Safety, Beijing Technology & Business University, Beijing, China*

[b] *Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Laboratory for Food Quality and Safety, Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

A R T I C L E I N F O

Article history:
Received 17 February 2017
Received in revised form 19 May 2017
Accepted 12 June 2017
Available online 13 June 2017

Keywords:
Lutein
DHA
Heteroaggregation
Emulsion
Microrheological property
Physicochemical properties
Oxidation

A B S T R A C T

The formation and physicochemical stability of mixed functional components (lutein & DHA) emulsions through heteroaggregation were studied. It was formed by controlled heteroaggregation of oppositely charged lutein and DHA droplets coated by cationic lactoferrin (LF) and anionic whey protein isolate (WPI), respectively. Heteroaggregation was induced by mixing the oppositely charged LF-lutein and WPI-DHA emulsions together at pH 6.0. Droplet size, zeta-potential, transmission-physical stability, microrheological behavior and microstructure of the heteroaggregates formed were measured as a function of LF-lutein to WPI-DHA droplet ratio. Lutein degradation and DHA oxidation by measurement of lipid hydroperoxides and thiobarbituric acid reactive substances were determined. Upon mixing the two types of bioactive compounds droplets together, it was found that the largest aggregates and highest physical stability occurred at a droplet ratio of 40% LF-lutein droplets to 60% WPI-DHA droplets. Heteroaggregates formation altered the microrheological properties of the mixed emulsions mainly by the special network structure of the droplets. When LF-coated lutein droplets ratios were more than 30% and less than 60%, the mixed emulsions exhibited distinct decreases in the Mean Square Displacement, which indicated that their limited scope of Brownian motion and stable structure. Mixed emulsions with LF-lutein/WPI-DHA droplets ratio of 4:6 exhibited Macroscopic Viscosity Index with 13 times and Elasticity Index with 3 times of magnitudes higher than the individual emulsions from which they were prepared. Compared with the WPI-DHA emulsion or LF-lutein emulsion, the oxidative stability of the heteroaggregate of LF-lutein/WPI-DHA emulsions was improved. Heteroaggregates formed by oppositely charged bioactive compounds droplets may be useful for creating specific food structures that lead to desirable physicochemical properties, such as microrheological property, physical and chemical stabilities.

1. Introduction

The bioactive compounds (e.g. carotenoids, omega-3 fatty acids, phytosterols etc.) have many positive effects on various physiological processes and are important to human's health (Ajilla, Anaidu, Bhat, & Prasada Rao, 2007). Therefore, bioactive compounds have been of interest to the pharmaceutical and food industries. Lutein is a yellowish pigment with two hydroxyl groups in the conjugated polyene chain (Sowbhagya, Sampathu, & Krishnamurthy, 2004). The functional properties of lutein are mainly recognized for reducing incidences of eye diseases such as age-related macular degeneration, reducing the risk of atherosclerosis, cancer and cardiovascular diseases (Mitri, Shegokar, Gohla, Anselmib, & Müllera, 2011). Previous studies have reported that the long chain docosahexaenoic acid (DHA) oil is highly beneficial to heart, brain, and nervous system functions and can prevent cardiovascular disease, immune disorders, inflammation, and some types of cancer

* Corresponding authors at: No. 11, Fucheng Road, Beijing 100048, China.
E-mail addresses: xuduoxia@th.btbu.edu.cn (D. Xu), xdxbtbu@126.com, caoyp@th.btbu.edu.cn (Y. Cao).

http://dx.doi.org/10.1016/j.foodchem.2017.06.078
0308-8146/© 2017 Elsevier Ltd. All rights reserved.

(Arab-Tehrany et al., 2012; Rubio-Rodríguez et al., 2010; Shahidi & Miraliakbari, 2004; Uauy & Valenzuela, 2000).

However, there are a number of oxidative stability issues of lutein and DHA that influence the product quality and consumer acceptance. The conjugated polyene chain of lutein and DHA are susceptible to degradation by oxidation and other chemical changes by exposure to air, light or heating during processing (Jacobsen, Bruni Let, Nielsen, & Meyer, 2008). The oxidation of lutein produces a number of initial oxidation products, such as carbon-peroxyl triplet biradicals and epoxides (Mesnier, Gregory, Fanca-Berthon, Boukobza, & Bily, 2014). The oxidation of DHA produces aldehydes, ketones, alcohols, hydrocarbons, hexanal, and propanal etc (Huber, Vasantha Rupasinghe, & Shahidi, 2009). The series of reactions could result in loss of both color and bioactivity of bioactive components in foods, lowering product quality and consumer acceptance.

There has been a growing interest within food and pharmaceutical industries in the development of different emulsion-based delivery systems to encapsulate, protect, and release various lipophilic bioactive food components (Dickinson, 2006; Lacatusu et al., 2013; Zhao, Cheng, Jiang, Yao, & Han, 2014). These delivery systems are to encapsulate single oil soluble component and the multiple bioactive food components delivery systems are limited. The potential application of heteroaggregates in the food industry is to encapsulate multiple oil soluble components. For example, positively charged emulsions can coat one kind of lipophilic component, whereas the negatively charged emulsions can be used to coat the other kind of lipophilic component. These two oppositely charged emulsions could then be mixed together to form microclusters to protect bioactive food components (McClements, 2012).

There were some studies on heteroaggregation between whey protein isolate coated droplets and modified starch or gum arabic coated droplets at low pH. At low pH values below the isoelectric point of protein, WPI coated droplets are positive, while the modified starch or gum arabic coated droplets are negative, leading to an electrostatic attraction between them (Schmitt & Kolodziejczyk, 2009). McClements et al. showed that materials with novel textural attributes could be created by mixing anionic β-lactoglobulin-coated lipid droplets with cationic lactoferrin-coated lipid droplets (Mao & McClements 2011; Mao & McClements 2012a). The rheological properties of these mixed emulsions have been shown to depend on the oil concentration, the ratio of different charged droplets, unadsorbed molecules, emulsifier exchange and aqueous phase conditions that decide electrostatic interactions, such as pH, ionic strength and temperature (Mao & McClements, 2013). The formation of a special three-dimensional network of aggregated droplets in the emulsion system led to elastic-like behavior. It can be used to create food products with novel textural characteristics, or it can be used to reduce the oil content of high fat food products (Dickinson, 2011; Maier, Zeeb, & Weiss, 2014). The special texture of the heteroaggregate was supposed to act as a physical barrier that separated bioactive components from prooxidants in the aqueous phase. However, the potential application of heteroaggregates in food industry to protect bioactive food components was not clear.

Whey protein is often utilized as an emulsifying agent and can retard the oxidation of dispersed phase by preventing the penetration of prooxidants in the emulsified droplet (Dickinson, 2008; Viljanen, Halmos, Sinclair, & Heinonen, 2005). Lactoferrin is a minor milk protein composed by a single polypeptide chain of about 80 kDa, containing from one to four glycans (Furtado, Michelon, Oliveira, & Cunha, 2016). Many studies have shown that lactoferrin is an excellent emulsifier since it adsorbs to the oil water interface and produces a cationic emulsion (Tokle & McClements, 2011; Ye & Singh, 2006). It also have many beneficial effects like antioxidant, antimicrobial, antiviral and anticancer activity (Actor, Hwang, & Kruzel, 2009; Tomita et al., 2009). Lactoferrin (LF) and whey protein isolate (WPI) at neutral pH were used to create droplets with different charges, since LF has an isoelectric point around 8 and is therefore positive, whereas WPI has an isoelectric point around 5 and is therefore negative (Mao & McClements, 2011). The LF-coated droplets and WPI-coated droplets have opposite charges when the pH is 6.0 and they will tend to associate with each other through electrostatic attraction.

In the current study, we examined the influence of positive-to-negative droplet (LF-lutein/WPI-DHA) ratio on the formation, physical (microrheology, physical stability and microstructure) and chemical properties (lutein and DHA oxidation) of mixed emulsions formed by heteroaggregation. We hypothesized that the heteroaggregation of oppositely charged LF coated lutein droplets and WPI coated DHA droplets might enhance the physicochemical properties of the emulsions. The knowledge gained from this study could be helpful for designing multiple functional components delivery system.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA). According to the manufacturer's specifications, the powdered WPI was constituted of 97.8% protein and 2% ash. Lactoferrin (LF) was purchased from Hilmar chemical co., LTD (Texas, USA). According to the manufacturer's specifications, the powdered LF was constituted of 93.2% protein, 0.01% iron, 0.0001% calcium and 0.02% potassium. Lutein powder (purity > 97%) was purchased from Baiyixingchen Pharmaceutical Technology Co., Ltd (Beijing, China). DHA rich algae oil was supplied by Jiabiyou Biological Engineering Co., Ltd (Wuhan, China). Medium-chain triglyceride (MCT) oil was obtained from Lonza Inc. (Allendale, NJ, USA). All other chemicals were of analytical grade.

2.2. Preparation of mixed emulsions by heteroaggregation

2.2.1. Single-protein emulsions (LF-lutein emulsion and WPI-DHA emulsion)

Single emulsions were prepared firstly. WPI and LF were first dispersed in the 1 mM phosphate buffer at pH 6.0, respectively. These solutions were kept overnight to ensure complete dispersion and dissolution, while sodium azide (0.02 wt%) was added to prevent microbial growth.

Lutein emulsion (3.5 wt% LF) was prepared with 10 wt% MCT oil containing lutein (0.06 wt% in the final emulsion, lutein was first dissolved in MCT oil at 140 °C for several seconds) as the dispersed phase and 90 wt% aqueous phase solution at room temperature. DHA emulsion (1 wt% WPI) was prepared with 10 wt% DHA oil as the dispersed phase and 90 wt% aqueous phase solution at room temperature.

The LF-lutein emulsion and WPI-DHA emulsion were then prehomogenized using an Ultra-Turrax (B25 model, Shanghai Beierte experimental equipment Co., Ltd) at a speed of 19,000 rpm for 3 min to form coarse emulsions, which were subsequently homogenized using a Microfluidizer processor (M-110PS model, Microfluidics International Corp., Newton, MA) at the operational pressure of 50 MPa three times, respectively. After preparation, the pH was adjusted to pH 6.0 using 1.0 M HCl or NaOH.

2.2.2. Mixed emulsions

The mixed emulsions were prepared by combining different mass ratios of emulsions containing LF-coated lutein droplets or