Food Hydrocolloids 72 (2017) 73–80

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Influence of NaCl on the oil/water interfacial and emulsifying properties of walnut protein-xanthan gum

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ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form
19 May 2017
Accepted 25 May 2017
Available online 26 May 2017

Keywords:
Walnut protein (WP)
Xanthan gum (XG)
NaCl
Interfacial properties
Emulsifying properties

ABSTRACT

Effect of NaCl concentration (0–500 mM) on the oil/water interfacial and emulsifying properties of walnut protein-xanthan gum complex (WP-XG) was investigated. In solution system, NaCl at 150–500 mM promoted the aggregation of WP-XG with a maximum hydrodynamic diameter of 553.1 nm. Compared with pure mixture, WP-XG solutions at 150–500 mM NaCl showed higher interfacial pressure and diffusion rate at the oil/water interface, and the maximum values, 18.05 mN/m and 0.431 mN m^{-1} $s^{-0.5}$ respectively, were obtained at 350 mM NaCl. With a maximum dilatational modulus of 48.47 mN/m at 150 mM NaCl, higher NaCl concentration would negatively influence the interfacial dilatational properties of WP-XG. In emulsion system, NaCl decreased ζ-potential, while increased surface protein concentration. According to the CLSM graphs, NaCl would aggravate depletion flocculation which was observed in pure emulsion. Large droplets were observed at 500 mM NaCl, with significant increase in mean particle diameter ($d_{4,3}$ = 2.90 μm).

1. Introduction

As an important type of food product, emulsions have been widely studied by food scientists. The stabilization of emulsion depends on the properties and the interaction between the oil droplets (McClements, 1999). In the range of 0.1–100 μm in diameter, the dispersion of the oil droplet in emulsions is in energetically unfavorable state due to the surface energy deficit (Lamorgese & Mauri, 2016; Lee, Choi, Li, Decker, & McClements, 2011). The interfacial tension, which equals the free energy per unit area of the interface between two phases (Gibbs, 2012), determines the size of oil droplets after homogenization. Amphiprotic emulsifiers, such as proteins, can adsorb at the interface and lower the interfacial tension to generate small oil droplets. Meanwhile, the interaction forces between oil droplets are critical for the stabilization during storage. A predominance of attractive forces (e.g. van der Waals and hydrophobic) leads to the destabilization due to droplet aggregation, while a predominance of repulsive forces (e.g. electrical and steric) leads to stabilization of the droplets. Adsorbed emulsifiers can form an interfacial layer around the droplets that protects them against destabilization, but the interfacial coatings formed by protein are usually relatively thin and vulnerable (McClements, 2004). The interactions of proteins and polysaccharides can be used to engineer the properties of the droplet interfaces (Zhao et al., 2015), and therefore improve emulsion stability.

Milk proteins, such as caseinates and whey protein, have been identified as efficient emulsifiers. However, due to craving for more sustainable sources and changing diet habit, there is considerable interest in identification of new emulsifiers. Recently, a study showed that walnut protein isolate exhibited good emulsifying properties and high surface hydrophobicity (276.51) (Mao & Hua, 2012), which was higher than soy protein isolate reported by Zhu et al., 2010. As one of the most popular dry products, walnut (*Juglans regia* L.) is commonly used in oil extraction (Vanhanen & Savage, 2006). However, walnut dregs produced by extraction are typical waste material used as feed or fertilizer. The composition of walnut dregs has been well studied, with a protein content of over 50% being reported. Moreover, walnut protein contains high levels (up to 80%) of glutelin and globulin in the protein fraction (Sze-Tao & Sathe, 2000). As China is the world's biggest producer and

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http://dx.doi.org/10.1016/j.foodhyd.2017.05.031
0268-005X/

consumer of walnut, it is profitable to determine the emulsifying properties of walnut protein (WP) so that it can be used as a value-added ingredient in foods and other industries. In particular, the utilization of WP-polysaccharide complexes may increase the scope of its utilization in a wide range of applications.

However, the adsorption of protein can be modulated or tuned by salt (e.g. NaCl, KCl, and $CaCl_2$, etc.) via the conformational change of protein and/or other ingredients in the solution (e.g. polysaccharide). More importantly, the electrostatic screening effect of salt would greatly deplete the electrostatic repulsion between droplets (Guzey & McClements, 2006). Due to its effects on both the properties and interactions of the oil droplets, salt could significantly influence the emulsion stability. However, saline reduction, on account of its vital role as protein stabilizer and acidity regulator, is hard to compromise. The construction of multilayer emulsion with magnified steric force is an applicable method to stabilize oil droplets under saline impact. A combination of protein and polysaccharide would promote protein adsorption at the oil/water interface and form a protein-polysaccharide complex membrane against ionic treatment (Akhtar & Dickinson, 2003).

As an anionic microbial polysaccharide with a molecular weight over 2×10^6 Da, xanthan gum (XG) is widely used in emulsions due to its viscosity and interaction with protein (Brunchi, Morariu, & Bercea, 2014). Numerous studies have been reported on the improved emulsifying effect of protein-XG complex (Liu et al., 2012; Zhao, Zhao, Yang, & Cui, 2009).

Therefore, the objectives of this paper were to a) study the interaction of WP-XG, b) assess the effect of NaCl concentration on the interfacial properties of WP-XG, and c) evaluate the change on the emulsifying properties of WP-XG. The adsorption behavior and the interfacial properties of WP-XG at the oil/water interface at different NaCl concentrations, and the stabilization evaluation of the emulsions were analyzed.

2. Materials and methods

2.1. Materials

WP was self-manufactured from walnut dregs by alkali extraction of sodium hydroxide and acid precipitation of hydrochloric acid, with the following compositions: 87.9 wt% protein (N × 5.3), 4.62 wt% ash, 4.12 wt% water and 3.12 wt% fat. The isoelectric point of WP is 4.45. XG was purchased from Zhongxuan Inc. (Shandong, China). Nile blue and Nile red of technical grade were obtained from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, USA). Corn oil was purchased from local supermarket. All other reagents were of analytical grade.

2.2. Preparation of WP-XG mixtures

Stock solutions of WP (2.4 wt%) and XG (1 wt%) were dissolved in phosphate buffer solution (PBS, 10 mM, pH 7.0), then stirred at a moderate speed at room temperature for 3 h for complete hydration. A WP-XG mixture containing 1.2 wt% WP plus 0.2 wt% XG was prepared and stirred for 2 h for homogeneous distribution. A WP solution (1.2 wt%) was prepared for hydrodynamic diameter measurement. NaCl was added into WP-XG mixture and WP solution to a final concentration of 0, 50, 150, 250, 350 and 500 mM respectively, and the corresponding ionic strength was 0, 50, 150, 250, 350 and 500 mM respectively.

2.2.1. Hydrodynamic diameter

The hydrodynamic diameter (D_H) was determined by dynamic light scattering via a NANO ZS Malvern Zetasizer (Malvern Instruments Ltd., UK) equipped with a He-Ne laser (wavelength of 633 nm) according to the method described by Carneiro-da-Cunha, Cerqueira, Souza, Teixeira, & Vicente, 2011. After diluted by PBS to a protein concentration of 0.1 wt% and a 1-min equilibrium time, stock solutions were subjected for measurement at 25 °C under a detector angle of 173°. Each measurement was carried out in triplicate.

2.3. Measurement of interfacial properties

The influence of NaCl concentration on the interfacial pressure and the dilatational parameters was determined according to the method of Caseli, Masui, Furriel, Leone, & Zaniquelli, 2005 using an optical contact angle meter DSA100 (KRUSS GmbH Ltd., Germany), with a DS3265 module for interfacial rheology. Samples were diluted by PBS into a protein concentration of 0.1 wt% before measurement.

2.3.1. Purification of corn oil

As a slight amount of surface active ingredients in corn oil could shed influence on the measurement of surface tension, corn oil was purified ahead according to the method of Liu et al., 2012.

2.3.2. Interfacial pressure

After thermostabilization process, a pendant drop of sample was injected by a syringe into an optical glass cuvette filled with purified corn oil. The controlled injection was to form an intact droplet with certain volume. The droplet image was captured periodically by a CF3217 high-speed camera and digitized for calculation of interfacial pressure. The sampling frequency was once per second for the first 1500 s, and then was once per 5 s afterwards. Each measurement was conducted at 25 °C for 10800 s.

The kinetic parameters for dynamic adsorption were calculated from the interfacial pressure (π) according to the method described by Liu, Zhao, Liu, & Zhao, 2011. The initial increase in π value was quantitatively related to the diffusion rate (K_{diff}), while the continuous rise was used for the calculation of the penetration rate (K_P) and the rearrangement rate (K_R), indicating the slow unfolding and rearrangement of protein after diffusion.

2.3.3. Interfacial dilatational properties

A sinusoidal oscillation with a frequency of 0.1 Hz and an amplitude ($\Delta A/A$) of 10% (within the linear region) was applied on the droplet in 2.3.2. Under the compression and expansion on the droplet, interfacial tension (σ) and surface area (A) were determined from the image contour, and the dilatational modulus (E) was calculated from the variation of σ and the variation of the natural logarithm of A (Lucassen & Tempel, 1972). The angle difference between σ and A was defined as the phase angle (θ) between stress and strain. Each measurement was conducted at 25 °C for 10800 s.

2.4. Preparation of emulsions

With the addition of 10 wt% corn oil into WP-XG mixtures, the new mixtures were sheared at 10,000 rpm for 1 min at room temperature by high speed homogenizer Ultra-Turrax T25 (IKA Ltd., Germany). Homogeneous emulsions were made by further passing through a two-stage valve homogenizer (APV-1000, Albertslund, Denmark) at 30 MPa. The emulsions were stored in refrigerator at 4 °C with sodium azide (0.02 wt%) for antisepsis.

2.5. Measurement of emulsion properties

2.5.1. Flow behavior

The fluid properties of emulsions were measured at 25 ± 1 °C by