LWT - Food Science and Technology 65 (2016) 676–682

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Studies of phase separation in soluble rice protein/different polysaccharides mixed systems

Xianghong Li*, Yongle Liu, Na Li, Ding Xie, Jian Yu, Faxiang Wang*, Jianhui Wang

Department of Food and Biological Engineering, Hunan Provincial Engineering Technology Research Center of Aquatic Food Resources Processing, Changsha University of Science & Technology, 960 South Wanjiali Road, Changsha 410114, Hunan Province, PR China

ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form
17 August 2015
Accepted 25 August 2015
Available online 28 August 2015

Keywords:
Soluble rice protein
Phase separation
Depletion
Microstructure

ABSTRACT

In present study, phase separation of soluble rice protein (SRP) and different polysaccharides were used to create specific structures with various structural and textural properties. The phase behavior and phase separation mechanism of mixtures of SRP and κ-carrageen/dextran (25 °C, pH 7.0 and 0.1 mol/L NaCl) were investigated by means of phase diagram construction, confocal laser scanning microscopy (CLSM) and stress-controlled rheometry. Phase diagrams combined with CLSM indicated that entropic effects of depletion interaction were the main driving force behind the phase separation of soluble rice protein and polysaccharides. Results also demonstrated that electrostatic interaction played an important role in the observed phase separation. CLSM and frequency sweeps revealed the effective association among rice proteins and formation of network-like structures in the mixed systems. These findings suggest that controlled phase separation and gelation of mixed SRP/polysaccharide systems have potential to create rice protein foods with novel textural characteristics.

1. Introduction

The incidence of individuals with celiac disease (a lifelong autoimmune disorder that is passed on genetically triggered by the ingestion of proteins of wheat, rye, and barley in genetically susceptible individuals) comprises approximately 1% of the worldwide population (Catassi & Fasano, 2008). Wheat is one of so-called big eight foods associated with allergenic reactions (Foegeding & Davis, 2011). Therefore, much more new strategies are needed to create gluten-free foods with desirable physical and sensory qualities. Rice is regarded as one of the most appropriate cereal grains for producing gluten-free products owing to benefits of rice proteins, which have highly nutritional properties (rich in the essential amino acid lysine) and functions such as hypoallergenic, hypocholesterolemic (Chrastil, 1992), antioxidative properties (Kawase, Matsumura, Murakami, & Mori, 1998) and anti-cancer activities (Xia et al., 2012). Moreover, recent researches revealed that rice proteins can be extracted from broken or debris rice (Li et al., 2010), rice bran (Tang, Hettiarachchy, Eswaranandam, & Crandall, 2003) or by-product of rice syrup production (Zhao et al., 2012), which are all inexpensive, underutilized co-products of rice products.

However, in spite of all the advantages, rice proteins have relatively poor functional properties for food processing. Due to their high content of glutelin (about 80%), rice proteins are insoluble in neutral aqueous solution. And low content of prolamins (about 3%) results in the lack of formation of a viscoelastic protein network (Hamaker, 1994). As a consequence, the rice protein products would exhibit low specific volume and very compact crumb. Therefore, addition of strengthening agents and specific treatments such as fermentation, hydrothermal and enzyme treatments have been proposed to provide necessary network for processing of rice products (Gujral, Guardiola, Carbonell, & Rosell, 2003; Hormdok & Noomhorm, 2007; Yang & Tao, 2008).

One of the easiest ways to generate protein gel structures with a range of textural and water-holding properties is to take advantage of the interactions of protein with other polymers. Nowadays, the use of phase separation of protein-polysaccharide mixtures to change food properties has received considerable attentions (Çakir & Foegeding, 2011; Chung, Degner, & McClements, 2013; Rohart & Michon, 2014).

Our previous studies demonstrated that the interaction of acid-deamidated rice protein and dextran can induce the formation of a protein network-like structure in the mixture with higher protein and/or polysaccharide concentration. Thermodynamic

Abbreviations: CLSM, confocal laser scanning microscopy; SRP, soluble rice protein.

* Corresponding authors.
E-mail addresses: xianghongl@163.com (X. Li), wfaxiang@163.com (F. Wang).

http://dx.doi.org/10.1016/j.lwt.2015.08.064
0023-6438/

incompatibility induced segregative phase separation was possibly favored under that condition and consequently promoted the self-association of protein molecules (Li et al., 2010). But the phase behavior and phase separation mechanism of the rice protein/polysaccharide system are still unknown. The overall texture and stability of food products also depend on the nature and strength of protein/polysaccharide interaction (Hemar, Hall, Munro, & Singh, 2002). Therefore, knowledge of the behavior and mechanisms of phase separation occurring in rice protein-polysaccharide systems is important in developing desirable properties in food products.

As far as we are aware, phase separation of rice protein and polysaccharides has not yet been investigated, and there are few investigations that have designed to obtain specific structures using systems of rice protein and polysaccharides. The present work has been focused on (i) producing soluble rice protein samples with different size by glutaminase-deamidation of rice protein to evaluate their effects on the interaction with different polysaccharides (κ-carrageenan and dextran); (ii) describing the phase behavior of the mixtures in terms of the location of the coexistence curve using visual inspection, at 25 °C, pH 7.0 and 0.1 mol/L NaCl; (iii) investigating the microstructures and rheological properties of several mixtures by CLSM and stress-controlled rheometry. Mechanism of phase separation related to the mixtures has also been discussed.

2. Materials and methods

2.1. Materials

Broken and debris rice, which was by-product of polished rice, was supplied by Hunan Grain Group. The rice has protein content of 8.2 wt% (N × 5.95, dry base). Polysaccharides (κ-carrageenan and dextran) were purchased from Sigma Co. (St Louis, MO, USA). All other reagents and chemicals were of analytical grade.

2.2. Preparation of different soluble rice protein samples

Soluble rice protein samples were prepared based on our previous study (Liu et al., 2011). The samples, which were glutaminase deamidated in 200 mmol/L sodium phosphate buffer (pH 7.0) containing 10 mg/mL rice glutelin and 0.1 unit/mL glutaminase for 2 h and 20 h (SRP_2 and SRP_{20}), were centrifuged at 10,000 r/min for 15 min, and the supernatants were dialyzed against 0.1 mol/L acetic acid, and then against deionized water at pH 7.0. Then the solutions were laid in dialysis bags (3.5 kD) and concentrated by directly placing onto a bed of polyethylene glycol (20,000 g/mol) chips for 6 h at 4 °C (Walsh, 2014). The protein content of the final solutions was determined according to the method of Lowry, Rosebrough, Farr, and Randall (1951). The protein samples were also characterized and used as representative samples of phase separation.

2.3. Static laser light scattering, intrinsic viscosities and zeta potential measurements

SRP_2 and SRP_{20} were characterized using static laser light scattering, intrinsic viscosity and zeta potential. A commercial laser light scattering spectrometer (ALV/DLS/SLS-5022F, Germany) was used, which was equipped with an ALV-5000/EPP multi-t digital time correlator and a He–Ne laser (Uniphase, output power ≈ 20 mW and $\lambda = 632.8$ nm). The static light scattering experiments were carried out at 25 ± 0.1 °C for at least three times (Li et al., 2009). The radii of gyration (i.e. the root mean square radius of particle and a constant correlating with particle size of protein) and weight-average molecular weights of two kinds of soluble rice protein samples were obtained.

The intrinsic viscosities were determined by dilute solution viscometry using a Ubbelohde glass viscometer (0.7–0.8 mm, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) in a constant temperature water bath at 25 ± 0.1 °C according to the method of Li, Cui, Wang, and Yada (2011) with modification. The measurements were made on a concentration range so that the relative viscosity, η_r ($=t/t_0$), was kept from 1.2 to 2.0 and the viscosity was essentially Newtonian. Then Huggins–Kramer plots of η_{sp}/C and $\ln(\eta_r)/C$ versus C were used to estimate the intrinsic viscosity [η] by extrapolation to zero concentration. Where C is concentration of protein and η_{sp} is specific viscosity, defined as $\eta_r - 1$. The averages of six measurements were used to calculate the intrinsic viscosities.

The zeta potentials of the samples were determined using a Zetasizer Nano ZS90 (Malvern Instruments, Southborough UK). The sample dispersions were diluted by a factor of 10^5 with distilled water. The samples were injected into the apparatus and the averages of five measurements were reported as zeta potential (Liu et al., 2011).

2.4. Preparation of polysaccharide solutions

The κ-carrageenan (κ-car) solution was prepared as follows: the powders were suspended in distilled water and stirred for 2 h at 70 °C. The pH was adjusted to 9 to eliminate the risk of hydrolysis during the preparation. The solution was first dialyzed against deionized water at pH 7.0 to eliminate excess salt and subsequently against 0.1 mol/L NaCl, pH 7.0. In 0.1 mol/L NaCl solution κ-car is negatively charged above 11 °C (Piculell, 1995). During the dialysis, the buffer solutions were changed for many times in 24 h. The final solution was filtered through a cellulose acetate membrane with pore size of 0.45 μm (Merck, Germany) to remove any insoluble particles.

The dextran solution was prepared as follows: the powders were suspended in distilled water and stirred for 2 h at room temperature. The pH was adjusted to 7.0 with 0.01 mol/L NaOH. The final solution was filtered through cellulose acetate membranes with pore size of 0.45 μm (Merck, Germany) to remove any insoluble particles.

The concentration of both polysaccharides was determined by phenol/sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The radii of gyration of κ-car and dextran were observed by static laser light scattering (Li et al., 2011).

2.5. Determination of phase diagrams

Phase diagrams of the mixtures were described in terms of the location of the coexistence curves using visual observations of a large number of mixtures with different concentration of SRP and polysaccharides, at 25 °C, pH 7.0 and 0.1 mol/L NaCl. The mixtures were prepared as follows: κ-car and dextran solutions were added to SRP solutions (SRP_2 and SRP_{20}) with different ratios at pH 7.0 and stirred thoroughly with vortex-stirrer at room temperature in 10 mL glass test tubes. The NaCl were also added to the mixtures to keep the concentration of 0.1 mol/L. And 0.02 wt% NaN_3 was used to avoid bacterial growth. The mixture solutions were degassed in a vacuum chamber until the visible bubbles were removed. The mixtures were then hermetically sealed and placed in a 25 ± 0.1 °C water bath for at least 24 h, after which the occurrence of phase separation (or not) was verified by visual inspection. A mixture was determined to be a gel when the mixture solution was transformed into a solid based on the visual observation. A spatula was used to tip the mixture to detect the transition from fluid to solid (Zhang & Foegeding, 2003). During all the experiments, the concentration of protein in each mixture was determined according to the method of Lowry et al. (1951), and the polysaccharide concentration was