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Effect of limited enzymatic hydrolysis on structure and emulsifying properties of rice glutelin

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ABSTRACT

The influence of the degree of hydrolysis (DH) on the structure, solubility, rheology, and emulsifying properties of rice glutelin was investigated. Structural properties were characterized using analysis of molecular weight distribution, surface hydrophobicity, intrinsic fluorescence, and Circular Dichroism (CD) spectra. Protein hydrolysis changed molecular weight, increased flexibility, altered surface hydrophobicity, and increased solubility. Oil-in-water emulsions were prepared from rice glutelin with different DH (0.5%, 2%, and 6%) and their stability to storage, pH, salt, and thermal processing was assessed. The storage, pH, salt, and temperature stability of the emulsions increased with decreasing hydrolysis. Emulsions prepared with 2% DH rice glutelin were stable over a range of environmental conditions: pH 7–9; NaCl <100 mM (pH 7); temperatures < 90 °C (pH 7, 0 mM NaCl) studied. These results will facilitate the formulation and production of natural emulsion-based products using rice glutelin as an emulsifier.

1. Introduction

The nature of the emulsifier used to formulate oil-in-water emulsions plays an important role in determining their structural, physicochemical and functional properties, such as droplet size, charge, interactions, stability, rheology, appearance, and digestibility (McClements, 2015). A wide variety of emulsifiers is available for utilization within the food industry, including synthetic surfactants, proteins, polysaccharides, and phospholipids (Kralova & Sjoblom, 2009). Nevertheless, there is currently considerable interest in the utilization of natural emulsifiers in foods due to consumer demands for natural products and clean labels (Ozturk & McClements, 2016). Many types of food proteins are amphiphilic molecules that can be used as emulsifiers to stabilize food emulsions (E. Dickinson, 2003, ; Eric Dickinson, 2011; Qian, Decker, Xiao, & McClements, 2011; Qiu, Sun, Zhao, Cui, & Zhao, 2013). The physicochemical and functional properties of emulsions depend on the interfacial characteristics of the protein molecules at the droplet surfaces, *e.g.*, adsorption kinetics, surface load, packing, thickness, electrical charge, rheology, and hydrophobicity (E. Dickinson, 2003; Murray, 2002). At present, the majority of protein-based emulsifiers used in the food industry are derived from dairy sources *e.g.*, whey proteins and caseins (Adjonu, Doran, Torley, & Agboola, 2014; Ozturk & McClements, 2016; Wilde, 2009). However, there is interest in developing plant-based alternatives to these proteins due to allergenicity, economic, sustainability and dietary issues (Karaca, Low, & Nickerson, 2015; Lam & Nickerson, 2013; Ozturk & McClements, 2016).

In this study, we focused on the emulsifying properties of proteins derived from rice, a highly abundant staple food. Rice protein is rich in essential amino acids, has a high nutritive value, lowers cholesterol, and is hypoallergenic (Du et al., 2013; Qiang; Zhao, Selomulya, et al., 2012). Previously, rice proteins have been reported to have good emulsifying properties under acidic conditions

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(Romero et al., 2012), but not under neutral conditions (Liu et al., 2011), which was attributed to differences in the aggregation state of the proteins under different pH conditions. The functional properties of proteins can often be improved by modification of their molecular structure and conformation using physical treatments (such as heating (Tang & Ma, 2009) or sonication (L. Jiang et al., 2014)), chemical treatments (such as deamidation (Paraman, Hettiarachchy, & Schaefer, 2007) or glycosylation (Du et al., 2013; Li et al., 2009)), or enzymatic treatments (Liu et al., 2011; Zheng et al., 2015). Moderate heating has been shown to significantly enhance the functional properties of vicilin-rich protein isolates (such as solubility, emulsifying activity, and foaming activity), while excessive heating has been shown to reduce these properties (Tang & Ma, 2009). Enzymatic treatments are often preferred for this purpose due to the mild reaction conditions required, and the high degree of specificity in altering molecular properties (Guan, Yao, Chen, Shan, & Zhang, 2007). In the current study, we focused on using hydrolytic enzymes to alter the degree of hydrolysis (DH) of rice glutelin so as to improve its solubility and emulsifying properties.

A number of previous studies have examined the factors that influence the emulsifying properties of rice protein hydrolysates. Studies have shown that combining rice protein hydrolysates (DH = 7.8%) with a non-ionic surfactant (Tween 20) can lead to the formation of more stable emulsions than possible with the protein hydrolysates alone (Qiang Zhao, Xiong, et al., 2012). Moreover, these combined rice protein hydrolysates/Tween 20 systems have been shown to have good antioxidant activity in emulsions (Cheetangdee & Benjakul, 2015). However, the utilization of a synthetic surfactant to improve the functional attributes of rice protein hydrolysates is undesirable for products that required an all-natural label. Some studies have shown that rice protein hydrolysates can adsorb to oil droplet surfaces but that they are not very effective in preventing coalescence, which may be because the interfacial layers formed are relatively thin and mobile (Adjonu et al., 2014; Lam & Nickerson, 2013). In general, emulsion stability depends on the effectiveness of the emulsifier at producing small droplets during homogenization, and then preventing their aggregation during storage or when environmental conditions are changed (McClements, 2015; Qian et al., 2011).

The ability of rice glutelin to form and stabilize emulsions is likely to depend on the degree of hydrolysis, since this will affect the molecular and functional characteristics of the hydrolyzed proteins. We therefore compared the influence of different degrees of hydrolysis on the molecular weight distribution, conformation, surface hydrophobicity, and functionality (solubility, rheology, and emulsifying properties) of rice glutelin. In addition, the impact of different environmental conditions (pH, ionic strength, and temperature) on the stability of the emulsions formed was examined so as to elucidate conditions where the proteins could be effectively used as ingredients in foods. This study is important for the identification of natural hypoallergenic protein emulsifiers that can be used in the development of label-friendly emulsion systems.

2. Materials and methods

2.1. Materials

Rice protein (90.3 wt%, dry basis) was kindly provided by Golden Agriculture Biotech Company Limited (Jiangxi, China). Sodium chloride, sodium azide, trypsin, and other chemicals were of analytical grade and were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO). Double-distilled water was used throughout to prepare all solutions and emulsions.

2.2. Extraction of rice glutelin

Rice glutelin was extracted from rice protein based on its differential solubility in different solutes using a procedure described previously (Ju, Hettiarachchy, & Rath, 2001; Likitwattanasade & Hongsprabhas, 2010). Rice protein was washed with 5% NaCl solution, 70% ethanol and distilled water until no protein was detected in the washing liquor. The final glutelin was recovered by centrifugation at 3000*g* for 30 min, freeze-dried, and stored at −20 °C. The protein content of rice glutelin was 93.42% (dry basis) as determined by the Kjeldahl method (N% × 5.95) (Qiang Zhao, Selomulya, et al., 2012).

2.3. Preparation of rice glutelin hydrolysates

Rice glutelin was hydrolyzed by trypsin at an optimized enzyme/substrate ratio according to preliminary experiments. 2 g of rice glutelin was dispersed in 30 mL deionized water and stirred for 1 h. After pH and temperature adjustments (pH = 8, 50 °C), Trypsin was added. Inactivation of the enzyme was achieved by heating to 95 °C for 10 min followed by immediate cooling in water to room temperature. The mixture was adjusted to pH 7.0, followed by centrifugation (TGL-20B, Anting Scientific Instrument Factory, Shanghai, China) at 4800 g for 10 min and the supernatant was freeze-dried. The hydrolysates formed had 0.5%, 2% and 6% DH, and were therefore referred to as DH0.5RG, DH2RG and DH6RG, respectively.

2.4. Estimation of the degree of hydrolysis

The degree of hydrolysis (DH), defined as the number of hydrolyzed peptide bonds (h) relative to the number of peptide bonds per unit weight (h_{tot}) expressed as a percentage, was determined by using the pH-stat method (Adler-Nissen, 1986) and calculated according to the following equation:

$$DH = \frac{h}{h_{tot}} \times 100\% = \frac{BN_b}{\alpha h_{tot} M_p} \times 100\% \quad (1)$$

Where B is base consumption (mL), N_b is the molarity of the base used, M_p is the mass (g) of the protein, h_{tot} is the theoretical overall number of peptide bonds in the protein substrate (7.40 meq/g rice protein) (Qiang Zhao, Xiong, et al., 2012), and α is the average degree of dissociation of the α-NH_2 groups expressed as:

$$\alpha = \frac{10^{pH-pK_a}}{1 + 10^{pH-pK_a}} \quad (2)$$

Here pH is the value at which the enzyme hydrolysis was conducted, and pK_a is the average pK_a for α-NH_3^+ groups on the protein. The value of α was 0.885 at pH 8 and 50 °C treatment (Adler-Nissen, 1986; García-Moreno et al., 2014).

2.5. Structural characteristics of hydrolyzed rice glutelin

2.5.1. Molecular weight distribution

A high performance size exclusion chromatography (SEC-HPLC) system (Agilent 1260 series) equipped with an appropriate column (Shodex KW804) was used according to the methods described previously (Liu et al., 2011) with some modification. Protein samples were dissolved in 100 mM phosphate buffer (pH 7.0) containing 20 mg/mL SDS. The supernatants were collected after centrifuged at 4800 g for 10 min. All protein solutions were filtered through 0.45 μm cellulose acetate membranes (Millipore, Billerica, MA) before use. The eluent used was 200 mM phosphate buffer