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Effectiveness of partially hydrolyzed rice glutelin as a food emulsifier: Comparison to whey protein

Xingfeng Xu [a,b], Junzhen Zhong [a], Jun Chen [a], Chengmei Liu [a,1,*], Liping Luo [a], Shunjing Luo [a], Lixin Wu [c], David Julian McClements [b,d,1,*]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, No. 235 Nanjing East Road, Nanchang 330047, Jiangxi, China
[b] Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA
[c] Golden Agriculture Biotech Company Limited, No. 100 Xinzhou Road, Nanchang 330000, Jiangxi, China
[d] Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia

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ABSTRACT

The emulsifying properties of partially hydrolyzed rice glutelin (H-RG, 2% degree of hydrolysis) were compared to those of whey isolate protein (WPI), a commonly used protein-based emulsifier. The surface load of WPI (1% emulsifier, d_{32} = 167.5 nm) was 2.8 times lower than that of H-RG (3% emulsifier, d_{32} = 159.0 nm). Emulsions containing WPI-coated lipid droplets had better stability to pH changes (2–8), NaCl addition (0–500 mM) and thermal processing (30–90 °C, 0 or 200 mM NaCl). Nevertheless, H-RG emulsions were stable over a range of conditions: pH 6–8; NaCl ≤ 200 (pH 7); temperatures ≤ 90 °C in the absence of salt (pH 7); and temperatures ≤ 50 °C in the presence of 200 mM NaCl (pH 7). This study indicates that H-RG may be utilized as a natural emulsifier in the development of label-friendly emulsion-based food products, but that further work is needed to increase the range of applications.

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1. Introduction

Many kinds of commonly consumed food products are emulsions, such as dairy creamers, dressings, dips, sauces and soft drinks (Dickinson, 2003). Emulsifiers are one of the most important additives for creating stable food emulsions. There are two major categories of emulsifiers used in the food industry: small-molecule surfactants (*e.g.,* Tween-80, monoglycerides, and lecithin) and large-molecule surfactants (*e.g.,* protein and polysaccharides) (Charoen et al., 2011; Ozturk, Argin, Ozilgen, & McClements, 2015). Nowadays, consumers are becoming more interested in having food products with "clean" labels, which can be achieved by replacing synthetic or animal-based emulsifiers with plant-based ones (Ozturk et al., 2015).

Food proteins are effective emulsifiers, because of their amphiphilic nature, relatively large molecular dimensions, and relatively high electrical charge (Dickinson, 2003; McClements, 2004). Consequently, they can adsorb to oil droplet surfaces during homogenization, and form coatings that protect droplets against aggregation by generating strong repulsive interactions (*e.g.,* steric and electrostatic repulsion). Whey proteins and caseins are the most commonly used protein-based emulsifiers in foods (Khouryieh, Puli, Williams, & Aramouni, 2015). However, because of their potential allergenicity (Do, Williams, & Toomer, 2016) and their unsuitability for utilization in vegan products, there is a demand for more label friendly plant-based alternatives. Rice glutelin (RG) is the major storage protein in rice, which is an attractive candidate for a plant-based emulsifier, because of its low allergenicity, ability to reduce cholesterol levels, and relatively low price (Agboola, Ng, & Mills, 2005; Chrastil, 1992; Fiocchi et al., 2003). Nevertheless, the application of native RG, as an emulsifier, is limited because of its low water-solubility and poor surface activity. Therefore, the native protein must be modified to improve its solubility and emulsifying properties. Enzyme modification has proved to be an effective means of altering the molecular, physicochemical and functional properties of proteins (Avramenko, Low, & Nickerson, 2013; Chen, Chen, Ren, & Zhao, 2011). Limited enzyme hydrolysis of rice protein has been shown to improve its emulsifying properties by increasing its surface hydrophobicity and molecular flexibility (Paraman, Hettiarachchy, Schaefer, & Beck, 2007). Recently, we examined the influence of the degree

* Corresponding authors at: Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA (D.J. McClements).
E-mail addresses: jiaonanxuxingfeng@126.com (X. Xu), zhongjunzhen@163.com (J. Zhong), chen-jun1986@hotmail.com (J. Chen), liuchengmei@aliyun.com (C. Liu), lluo2@126.com (L. Luo), luoshunjing@aliyun.com (S. Luo), wulixin@gabchina.com (L. Wu), mcclements@foodsci.umass.edu (D.J. McClements).
[1] These authors contributed equally to this manuscript.

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of enzyme hydrolysis on the ability of rice glutelin to form and stabilize emulsions. We found that the optimum emulsifying properties were obtained for the degree of hydrolysis as 2%, and so a hydrolyzed rice glutelin (H-RG) ingredient with this level of hydrolysis was used in the current study.

In the current study, we compared the ability of H-RG to form and stabilize oil-in-water emulsions, and compared its performance to whey protein isolate (WPI), which is already a commonly used emulsifier in the food industry. In particular, we focused on the influence of environmental stresses that food products may experience during their manufacture, storage and utilization, such as pH, ionic strength and temperature. This study therefore provides valuable information about the potential utilization of natural emulsifiers in the development of label-friendly emulsion-based food and beverage products.

2. Materials and methods

2.1. Materials

Rice glutelin (93.42 wt%, dry basis) was kindly provided by Golden Agriculture Biotech Company Limited (Jiangxi, China). Whey protein isolate (WPI) was purchased from Davisco Foods International Inc. (Le Sueur, MN). As stated by the manufacturer, the protein content was 97.6% (dry basis). Corn oil was obtained from a commercial food supplier (Mazola, ACH Food Companies, Memphis, TN). Double-distilled water was used throughout to prepare all solutions and emulsions.

2.2. Preparation of rice glutelin hydrolysates

Hydrolyzed protein was prepared using trypsin at an optimized enzyme/substrate ratio according to Zhao et al. (2012). 2 g of RG was added to double-distilled water (30 ml) with constant stirring for 1 h. After pH and temperature adjustments (pH = 8, 50 °C), trypsin was added. The pH of the suspension was kept constant by the addition of 2.5 M NaOH during the entire period of hydrolysis. After 4.2 min of hydrolysis, the dispersion was heated to 95 °C for 10 min to inactivate the enzyme, followed by adjustment of the pH to 7.0 using HCl or NaOH, and immediate cooling in water to room temperature. The mixture was centrifuged (TGL-20B, Anting Scientific Instrument Factory, Shanghai, China) at 4800*g* for 10 min, and the supernatant was freeze-dried. The degree of hydrolysis (DH) was calculated from the amount of base required to maintain a constant pH using the pH-stat method described previously (Adler-Nissen, 1986):

$$\mathrm{DH} = \frac{h}{h_{tot}} \times 100\% = \frac{\mathrm{BN_b}}{\alpha h_{tot} \mathrm{M}_p} \times 100\%$$

where B is base consumption (ml); N_b is the molarity of the base; M_p is the mass (g) of the protein; the value of α was 0.885 at pH 8 and 50 °C treatment (Adler-Nissen, 1986; García-Moreno et al., 2014), and h_{tot} is the theoretical overall number of peptide bonds in the protein substrate (7.40 meq/g rice protein) (Zhao et al., 2012). Rice glutelin with degree of hydrolysis of 2% was finally obtained.

2.3. Characterization of rice protein and its hydrolysates

2.3.1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

The polypeptide profiles of samples were determined by SDS-PAGE, as described previously (Laemmli, 1970). Protein samples were dissolved in loading buffer that contained β-mercaptoethanol (Solarbio, China) and were heated in boiling water for 5 min, then cooled to room temperature. 20 μl of sample (1 mg/ml) was loaded on 5% stacking gel and 12.5% separating gel, and subjected to electrophoresis at a constant voltage of 80 V. Then the gels were stained for 2 h using 0.05% Coomassie Brilliant Blue R-250 in acetic acid/methanol/water solution (46:227:227, v/v/v) and destained for 6 h with methanol/acetic acid/water solution (50:75:875, v/v/v).

2.3.2. Water-solubility

The water-solubility of proteins was analyzed by measuring the protein content using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) with bovine serum albumin as a standard. 1 wt% of protein aqueous solution was stirred magnetically at 25 °C for 30 min, and the pH was then adjusted to the desired value using either 0.5 M HCl or 0.5 M NaOH. After 30 min of stirring, the pH was readjusted if necessary. The dispersions were centrifuged at 4800*g* for 15 min, and the protein content in the supernatant was analyzed. After appropriate dilution, the protein solubility was calculated as the percentage of protein in the supernatant relative to the total protein content. All measurements were conducted in triplicate.

2.4. Emulsion preparation

Protein solutions were prepared by dispersing 0.01–8 wt% WPI or H-RG into phosphate buffer (5 mM pH 7.0), and were stirred for at least 3 h. After overnight storage at 4 °C, protein solutions were filtered with a qualitative filter (Fisher Scientific, PA) to remove any insoluble components. The absorbance at 280 nm of the protein solutions was measured before and after filtration, which indicated that a small amount of protein (<2.5% of the total) was lost. 10 wt% oil phase (corn oil) and 90 wt% aqueous phase (protein in buffer) were homogenized using a high-shear mixer at 10,000 rpm for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). The coarse emulsions were passed through a high pressure homogenizer (M110Y, Microfluidics, Newton, MA), with a 75 μm interaction chamber (F20Y) and an operating pressure of 12,000 psi, for 3 times.

2.5. Emulsion stability

The stability of the emulsions was measured after they were subjected to environmental stresses that foods may experience during manufacture, storage and utilization.

2.5.1. pH

Freshly prepared emulsions (pH 7) were distributed into different beakers, and then each sample was adjusted to a different pH value, ranging from 2 to 8 using HCl or NaOH solutions, and then placed in test tubes.

2.5.2. Ionic strength

Freshly prepared emulsions (pH 7) were distributed into different beakers, and then each sample was adjusted to a different ionic strength by adding different amounts of NaCl solution, and then placed in test tubes.

2.5.3. Temperature

The temperature stability of freshly prepared emulsions (pH 7) was measured. Emulsions containing either 0 or 200 mM NaCl were transferred into test tubes, which were then heated in water baths set at different temperatures (30–90 °C) for 30 min. The test tubes were then cooled to room temperature by placing them in ice water.

After exposure to these environmental stresses, samples were stored at room temperature for 24 h prior to determination of their

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