Journal of Cereal Science 79 (2018) 204–209

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Influence of ultrasound-assisted alkali treatment on the structural properties and functionalities of rice protein

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ARTICLE INFO

Article history:
Received 8 April 2017
Received in revised form
28 August 2017
Accepted 17 October 2017
Available online 27 October 2017

Keywords:
Rice protein
Ultrasound-assisted alkali treatment
Solubility
Functional properties

ABSTRACT

The poor solubility of rice protein (RP) limits its applications in food industry. In this study, the effects of ultrasound-assisted alkali (UAA) treatment on the solubility, structure and functional properties of RP were investigated. Using UAA treatment, the solubility of RP increased with increasing alkali concentration, reaching a maximum value of 19.79 mg/mL at an alkali concentration of 0.08 M. The solubility was improved by 230-fold compared to un-treated samples. In addition, a reduction in particle size and degradation of the protein subunit were observed. UAA seemed to unfold the protein internal structural conformation and expose buried functional groups, which are linked to good emulsifying properties and foaming properties. A decrease in zeta potential was also observed after UAA treatment, which could be the reason for the decreased stability of the emulsion. UAA treatment modified the protein structure and significantly improved solubility.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in developing countries. It can provide 35%–59% of total caloric intake for more than 50% of the world's population (Juliano, 2016). In addition, rice is also an important raw material of the sugar industry because of the high starch content. After processing, about 50% of the raw rice becomes a byproduct called rice residue. In China, 10 million tons of rice residues were produced in the sugar industry every year. After the saccharification of starch, the protein content in the rice residue is increased to more than 50%. Because of the hypoallergenic properties and anti-cancer activity (Helm and Burks, 1996; Shoji et al., 2001), rice protein (RP) has gained increasing attention in the food industry. And the high content (66%–78%, w/w) insoluble glutenin resulted in the low solubility of RP (Wang et al., 2015a), which is a bottleneck for its extensive applications in food industry. Many treatments have been investigated to improve the solubility of RP. Various physical approaches, such as microfluidization (Xia et al., 2012a), hydrothermal cooking (Xia et al., 2012b) and high-pressure treatment (Kato et al., 2000) have been reported. Chemical methods, including enzymatic hydrolysis (Ahmadifard et al., 2016) and phosphorylation (Yi and Yao, 2005), have also been studied. Alkali solution is the most common means of extracting rice proteins, as the proteins show higher solubility in alkali media. However, the effects these treatments on the solubility of rice protein are still not satisfied.

Ultrasound has been used both to analyze food structure and composition at low ultrasonic intensities and high frequencies and to modify ingredients at high ultrasonic intensities and low frequencies. In the food industry, power ultrasound has proved to be a highly effective food processing and preservation technology, and use of high-intensity ultrasound with or without heat may be used, for example, to homogenize or disperse two-phase systems such as emulsions or suspensions (Mason et al., 1996). Ultrasound has also been tested for improving solubility and modifying the functional properties of proteins, which is considered an environmentally friendly and innovative technology. Ultrasound treatment damages the protein quaternary structure through cavitations, producing a small molecular subunit and increasing solubility of RP (Gulseren

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https://doi.org/10.1016/j.jcs.2017.10.013
0733-5210/

et al., 2007). Jambrak et al. (2008) reported ultrasound frequency of 20 kHz, power of 600 W and 30 min duration can improve solubility of protein.

Ultrasound assisted alkali treatment has also been reported in the modification of protein. According to the reported studies, in the ultrasound assisted alkali treatment, the protein solution's pH shifting to 12 or higher, compact structure of protein is getting loose, and the simultaneous ultrasound treatment damaged protein's structure easier and increased protein's solubility (Jiang et al., 2017; Lee et al., 2016; Yildiz et al., 2017).

To date, improving the solubility of RP via ultrasound assisted alkali treatment has not yet been investigated. In this study, rice protein powder with 90 wt% protein content extracted from rice residue was used as a raw material, and the effects of UAA treatment on the solubility as well as structural and functionalities of RP were investigated. Through these experiments, we aimed to evaluate the potential of UAA treatment for improving the application of RP in food industry.

2. Materials and method

2.1. Materials

RP (90 ± 1.13 wt% protein content, wet basis) were purchased from Jinnong Biotechnology Co. (Wuxi, Jiangsu, China). SDS-PAGE kit was purchased from Solarbio Co. (Beijing, China) and used without further purification. All of the other chemicals were of analytical grade and purchased from Sigma-Aldrich Co. LLC (Beijing, China).

2.2. Ultrasound assisted alkali treatment of rice protein

RP was dispersed in NaOH solutions with the following concentrations: 0.02, 0.04, 0.06, 0.08, and 0.1 M. The final protein concentration was 50 g/L (w/v) in each solution. A 100 mL aliquot of RP solution was transferred to a jacket beaker (250 mL) and treated by ultrasound at 50 °C for 60 min. The ultrasound experiments were carried out at 20 kHz using an ultrasound generator (Scientz Biotechnology Co., Ltd, Ningbo, China, Model: Scientz-950E) with a 12 mm vibrating titanium tip probe. The probe was immersed 2 cm into the liquid. The solution temperature was measured by the ultrasound generator built-in temperature sensors. The rating power of the ultrasound generator was 600 W and the ultrasound intensity was 19.3 W/cm^2 calculated as follows (Cárcel et al., 2007; Raso et al., 1999):

$$I_a = P_a/S_A, \text{where } P = m \cdot c_p \left(dT/dt \right) \quad (1)$$

where P_a (W) is the acoustic power, S_A is the surface area of the ultrasound emitting surface (1.13 cm^2), m is the mass of ultrasound treated solution (g), c_p is the specific heat of the medium (4.18 kJ/gK) and dT/dt is the rate of temperature change with respect to time, starting at t = 0 (°C/s).

After the UAA treatment, the samples were readjusted to pH 7.5, and centrifuged at 10,000 g for 15 min. Supernatants were collected and freeze-dried for 12 h.

2.3. Solubility measurements

Soluble protein in the supernatants was measured via the Bradford assay. Samples not subjected to ultrasound treatment served as the control. The effectiveness of the treatment was expressed as the accumulated concentration of the soluble protein content. Bovine serum albumin (BSA) was used as a standard for the Bradford assay. Absorbance at 595 nm was measured using a UV spectrophotometer (T6, Purkinje General Instrument Co. Ltd, Beijing, China). Protein solubility was expressed as the concentration of water-soluble proteins.

2.4. Emulsifying activity and emulsion stability

Emulsifying activity (EA) and emulsion stability (ES) were determined via turbidity measurements. A 1% (w/v) aqueous protein suspension was adjusted to pH 7.5 using 1 M hydrochloric acid. To 6 mL of the protein solution, 2 mL olive oil was added, and the mixture was homogenized in a mechanical superfine homogenizer (FA25, Fluko Equipment Shanghai Co., Ltd, China) at 10,000 r/min for 1 min to produce a full emulsion. After homogenizing, 50 μL aliquots of the emulsion were pipette at 0 min and 15 min and then mixed with 5 mL of 0.1% SDS solution respectively. The absorbance of the emulsion was measured at 500 nm with a UV spectrophotometer. The absorbance that was measured at time 0 min (T_0) was expressed as the EA of the proteins. The ES was determined as follows:

$$ES = T_0(\Delta t/\Delta T) \quad (2)$$

where ΔT is the change in turbidity and Δt is the time interval (15 min). BSA was used as the standard for emulsifying activity.

2.5. Foam activity and foam stability

The FA was expressed as the volume of foam immediately measured after foaming (10,000 r/min for 1 min) 40 mL of 1% protein solution containing 0.05 M phosphate buffer (pH 7.5) in a glass tube. The foam stability (FS) was calculated as follows:

$$FS = V_0(\Delta t/\Delta V) \quad (3)$$

where ΔV is the change in the volume of foam (V) occurring during the time interval Δt (30 min) and V_0 is the volume of foam at time 0 min. HEA was used as the standard for foaming activity.

2.6. SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method described by Laemmli (1970). SDS-PAGE was carried out on a gel slab comprised of 5% stacking gel and 15% separating gel in a SDS-Tris-glycine discontinuous buffer system. Protein powder was prepared in RRP (control) and different UAA-RRP (NaOH concentration 0.02, 0.04, 0.06, 0.08, and 0.1 M) conditions using a buffer solution with 2-mercaptoethanol. Electrophoresis was performed at a constant potential of 200 V per gel for approximately 2 h. The gels were stained with Coomassie Brilliant Blue R 250. The protein marker was purchased from Solarbio Co. (Beijing, China).

2.7. Sulfhydryl and disulfide bond contents

The free sulfhydryl group (SH_F), total sulfhydryl group (SH_T), and disulfide bond (S—S) content of the protein samples were determined according to the method described by Beveridge et al. (1974) with some modifications. RP (15 mg) was dissolved in 10 mL Tris—Gly buffer (pH 8.0) containing 0.086 M Tris, 0.09 M glycine, 0.004 M EDTA, and 8 M urea. The mixture was centrifuged at 10000g for 10 min. For SH_F content determination, 50 μL of Ellman's reagent (DTNB in Tris—Gly buffer, 4 mg/mL) was added to 1 mL of protein supernatant, and the solution was mixed. After reacting for 5 min, the absorbance at 412 nm was measured. For SH_T content