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Effects of ultrasonic and graft treatments on grafting degree, structure, functionality, and digestibility of rapeseed protein isolate-dextran conjugates

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ABSTRACT

Rapeseed protein isolate (RPI) and dextran conjugates were prepared by traditional and ultrasonic assisted wet-heating. The effects on the grafting degree (*GD*), structure, functionality, and digestibility of conjugates were studied. Ultrasonic frequency, temperature, and time all significantly affected the *GD*. Under the optimum conditions (temperature of 90 °C and time of 60 min), compared to traditional wet-heating, ultrasonic treatment at 28 kHz significantly increased the *GD* by 2.12 times. Compared to RPI, surface hydrophobicities of conjugates were significantly decreased by graft and ultrasonic treatments. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and amino acid composition results confirmed that traditional graft reaction involved cysteine (Cys) and lysine (Lys) whereas the ultrasonic assisted one involved only Cys. Both were from the 12S globulin subunit and cruciferin. Fourier transform infrared spectrum (FT-IR) and circular dichroism (CD) results showed that graft treatment significantly changed secondary structure and ultrasonic treatment had the greatest impact on the decrease in the β-sheet (19.1%) and the increase in the random coil (49.6%). Graft and ultrasonic treatments both made surface structure looser and more porous. The two treatments also caused molecular weight to become bigger, and ultrasonic treatment had the greatest effect on the increase (68.2%) in 110–20.5 kDa. Structural modifications of RPI by grafting to dextran caused improvements of solubility (at pH 5–6), emulsifying activity (at pH 4–10), emulsion stability (at pH 4–5 and 9–10), and thermal stability (at temperature 90–100 °C). The digestibility of conjugates was decreased by graft and ultrasonic treatments and the conjugates were mainly digested in the intestinal phase. The ultrasonic assisted wet-heating was an efficient and safe method for producing RPI-dextran conjugates and improving the utilization value of rapeseed meal.

1. Introduction

At present, rapeseed meal is the second most widely produced by-product as a consequence of increased oil extraction after soybean meal and is less expensive than soybean meal. According to reports, rapeseed meal has a high level of crude protein (320–400 g crude protein/kg) with a well-balanced amino acid profile and high S-containing amino acids [1–5]. Therefore, rapeseed is a good and abundant resource and has been recognized as a potential alternative protein source for human consumption [6]. Fleddermann et al. [7] found that rapeseed protein (both isolate and hydrolyzate) has a high nutritional quality and is considered as efficient as soy protein for a postprandial amino acid response. However, some functional properties of rapeseed protein are relatively low. Wanasundara et al. [8] reported that the solubility, emulsibility, foaming stability, and gel property of rapeseed protein are relatively poor. The digestibility of rapeseed protein is also low [9]. The functional properties of proteins are closely related to their structures. Therefore, to improve the functional characteristics of rapeseed protein, related research in structural modification method is needed.

The grafting of protein with sugar by means of the Maillard reaction has been proved to be a promising approach among various modifications through physical, chemical, and enzymatic treatments [10,11]. The Maillard reaction is generally regarded as a safe and efficient method to improve functional properties of proteins, such as emulsifying properties, solubility, antioxidant, anticarcinogenic, and antimutagenic activities [12–17]. The grafting of protein with sugar can be carried out by dry-heating, which always takes a long time (up to several days or weeks) to prepare Maillard reaction products and is uncontrolled [18,19]. To avoid this problem, the heating treatment is commonly done in an aqueous solution (wet-heating) to produce protein-sugar conjugates with special functional properties. Compared to dry-heating, the wet-heating method takes a short time (only several

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hours) and is more conducive to industrial production [19]. Nevertheless, protein-sugar graft reaction is time-consuming and the grafting degree is relatively low when using traditional heating treatment. Therefore, it is necessary to find a novel technology to improve the efficiency of graft reaction.

Ultrasound not only represents a rapid, efficient, and reliable alternative to shorten the time of graft reaction and increase the grafting degree, but it also has the potential to develop new products with a unique functionality [20]. Ultrasound can accelerate chemical reactions, increase diffusion rates, and disperse aggregates through acoustic cavitation [21]. The use of ultrasound in food industry has recently increased due to its promising effects in food and product modification. Compared with traditional wet-heating, ultrasonic assisted treatment can accelerate the graft reaction efficiency, and improve the solubility, emulsifying properties, and surface hydrophobicity of the conjugates [22,23]. However, there exist the following gaps in the research on ultrasonic treatment of the protein grafting. The first is that the systematic research has not been formed, including protein modification process optimization, structure change, and relevant functionality and digestibility improvements of grafted conjugates. At present, only a small amount of preliminary study in the physicochemical properties of protein-sugar conjugates prepared by ultrasonic treatment was reported [22,23]. The second is that the currently used ultrasound is still a static mode of ultrasonic processing. The advanced ultrasonic processing mode in protein modification such as countercurrent ultrasonic treatment used in this research has not been reported [24]. Under the countercurrent ultrasonic treatment, the direction of the ultrasonic field and material flow in the opposite directions, which can greatly increase the frequency of contact between ultrasound field and material and is more advantageous to unfold protein structure and improve *GD*. But the research on countercurrent ultrasonic treatment of the protein grafting has not been reported yet.

Dextran is generally used for the graft reaction with protein for two reasons. Dextran has a reducing nature, which is one of the required conditions for the reaction to take place. Secondly, its neutral charge inhibits the formation of electrostatic complexing between proteins and polysaccharides [25]. Many studies have shown that conjugates prepared by protein and dextran could significantly improve protein functionality, such as thermal stability, emulsifying activity, and emulsion stability [11,18,19,26,27]. However, there is no reported research regarding dextran modification of rapeseed meal protein isolates via ultrasonic assisted wet-heating method, and the studies of the structural and functional properties and digestibility of their conjugates.

The objectives of this study were to (1) investigate the sonochemical effect of ultrasonic factors on the *GD* of RPI-dextran conjugate compared to the traditional wet-heating method, and to (2) describe the effects of graft and ultrasonic treatments on the structural and functional properties and digestibility of RPI-dextran conjugates compared to RPI itself. This research was used to find a way to improve the applicability of rapeseed meal in the food industry by enhancing its functional properties.

2. Materials and methods

2.1. Materials and reagents

Rapeseed meal (60 mesh, crude protein content of 36.5% on dry basis determined by Kjeldahl method) was supplied from by-products of Zhengda Oil Co. Ltd. (Jiangsu, China). Dextran (MW 20 kDa) was ordered from Sinopharm Chemical Reagent Co. (Shanghai, China). Lys, L-leucine, bovine serum albumin (BSA), cytochrome *C*, glutathione, bacitracin, L-tryptophan, pepsin, trypsin, and 2,4,6 trinitrobenzene sulfonic acid (TNBS) were purchased from Sigma Company (St. Louis, MO, USA). Protein Molecular Weight Marker with MW 14.3–97.2 KDa was ordered from Takara Company (Dalian, China). HPLC-grade of acetonitrile was purchased from Tedia company (Fairfield, OH, USA). All reagents used in the experiment were of analytical grade.

2.2. Methods

2.2.1. Preparation of RPI

Rapeseed meal was extracted twice with 85% ethanol at a 1:4 ratio (w/v) and 50 °C for first 20 min and second 15 min. After centrifugation (3150*g*, 15 min) (TGL-16, Changsha Xiangyi Centrifuge Instrument Co. Ltd., Changsha, China), the two precipitates were collected. The precipitates were extracted twice with pH 12 lye at a 1:15 ratio (w/v) and 50 °C for 1 h each time. After centrifugation, the two supernatants were collected together and adjusted to pH 6 with 0.1 mol/L HCl. After centrifugation, the precipitate was collected. The supernatant was adjusted to pH 3.6 again. After centrifugation, the precipitate was collected again. Two RPI precipitates were dissolved in distilled water to dialyze at 4 °C for 24 h. The dialysate was freeze-dried (ALPHA1-2, Martin Christ, Osterode, Germany) to obtain the RPI material (crude protein content of 80%).

2.2.2. Preparation of RPI-dextran conjugates by traditional and ultrasonic assisted wet-heating

Firstly, the 4 mg/mL RPI solution (pH 10) was mixed with dextran at a 1:1 (w/w) ratio for 20 min. Subsequently, the RPI-dextran mixture (400 mL) was treated by the countercurrent ultrasound equipment manufactured by Jiangsu University [24] at 90 °C for 1 h under a power of 65.8 W/L and frequency of 28 kHz. The sonochemical effects of ultrasonic treatment on the *GD* were determined at different ultrasonic frequencies (20, 28, 35, 40, and 50 kHz), temperatures (70, 80, and 90 °C), and times (0, 10, 20, 30, 40, 50, and 60 min). The solutions without ultrasonic treatment were conducted as the traditional control. After reaction, the solution was immediately cooled down and dialyzed for 36 h at 4 °C. All RPI-dextran conjugates were collected for the determination of *GD*, respectively.

2.2.3. Digestibility tests

2.2.3.1. Gastric digestion. Gastric digestion test was performed according to the method described by Tang [28] with some modifications. The 10 mg/mL sample was adjusted to pH 1.5 and preheated at 37 °C for 5 min. Pepsin reacted with sample solution at 1:50 (w/w) ratio and 37 °C with a stirring speed of 100 rpm for 0, 10, 20, 30, 60, and 120 min, respectively. After digestion, the pepsin was inactivated at 100 °C for 10 min. The gastric hydrolysate was collected for the determination of nitrogen release rate (*NRR*).

2.2.3.2. Intestinal digestion test. Intestinal digestion test was conducted based on the description of Tang [28] with some modifications. Gastric hydrolysate, after a digestion time of 120 min, was adjusted to pH 7 and reacted with Trypsin at 1:50 ratio, 37 °C, and 100 rpm for 10, 20, 30, 60, and 120 min, respectively. After digestion, the trypsin was inactivated and the intestinal hydrolysate was collected for *NRR* determination.

2.2.4. Determination of GD

GD was determined according to the TNBS method [29] with some modifications. A volume of 0.4 mL 0.2% (w/v) conjugates solution prepared by 0.1% (w/v) SDS solution was reacted with 2 mL phosphate buffer (pH 8.2) and 1 mL 0.1% (w/v) TNBS for 30 min at 50 °C in the dark. Then, the reaction was immediately terminated by adding 2 mL 0.1 mol/L HCl. After standing for 30 min, the absorbance at 340 nm was read by a UV spectrophotometer (T6, Beijing Beifen-Ruili Analytical Instrument Co. Ltd, Beijing, China). *GD* was calculated as follows:

$$GD\ (\%) = (C_0 - C_t)/C_0 \times 100\% \quad (1)$$

where C_0 is the amino concentration of ungrafted sample (mmol/L), and C_t is the amino concentration of grafted sample at a grafting time *t*