



# Study on the mechanism of microwave modified wheat protein fiber to improve its mechanical properties



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## ABSTRACT

Wheat protein is widely used in food industry. In order to expand the scope of its application on non-food field, we managed to apply the wheat protein to fiber production. To improve the mechanical properties of the fibers, we used the method of microwave modification. The best process conditions obtained by response surface analysis were a microwave power of 20.6 W/mL, microwave time of 3 min, and pH 8. Compared to non-microwaved fibers, the breaking strength was 19% higher and the elongation was 302.43% higher which indicated the microwaved fiber toughness was increased. To study the mechanism underlying the effect of microwave treatment on the improvement of mechanical properties, changes in the –SH and –S–S– content during wheat protein fiber preparation, a secondary structure study, X-ray diffraction, thermal performance analysis, SEM, surface hydrophobicity, and standard moisture regain measurement were examined. The microwaved fiber had increased –S–S– content,  $\alpha$ -helices, crystallinity, which may be responsible for the better mechanical properties. DSC and TG results showed that the thermal stability of microwaved fiber was increased. Additionally, SEM micrographs revealed that the structure of microwaved fibers was smoother and denser, and contained less pores than non-microwaved fibers. Although the surface hydrophobicity and standard moisture regain were decreased, microwaved fiber had good hygroscopicity, which was close to that of silk.

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## 1. Introduction

Wheat gluten, the endosperm storage protein, is a typical water-insoluble protein that makes up about 85% of total wheat protein. Wheat gluten is formed by glutenin and gliadin through disulfide inter- and intra-molecule linkage (Shewry and Tatham, 1997). The unique properties required for forming flour dough make wheat gluten very popular in food industries, and also allow the development of biodegradable biomaterials (Veraverbeke and Delcour, 2002). Today, gluten is extensively used in human and pet food applications, but also used in non-food industries, such as natural adhesives, films for food packing, and so on (Day et al., 2006). Besides, there also have reports about wheat gluten fibers which have fineness similar to that of wool (Narendra Reddy and Yiqi Yang, 2007).

Wheat gluten is also a cheap, abundant, and renewable source for producing protein fibers (Lens et al., 1999; Ye et al., 2006; Woerdeman et al., 2004). In addition, it has good resistance to

water and heat, excellent elasticity, and easy degradability, all of which are desirable properties for fibers (Woerdeman et al., 2004; Bietz and Lookhart, 1996; Krull and Inglett, 1971). Therefore, the application of wheat gluten on fiber can not only expand its consumption market but also increase its value addition.

Several attempts have been made to use plant proteins such as soybeans, corn, and peanut or milk proteins (casein) for fiber production. However, pure plant protein fibers are scarce because of their poor properties. For example, soyprotein fibers' breaking tenacity is about 30% of wool and the breaking elongation is less than 10% of wool (Moncrieff, 1975; Farrow, 1956). Zein fibers' mechanical prosperity is similar to soyprotein fiber (Yang et al., 1996; Farrow, 1956). Modification of wheat gluten by physical, chemical, and enzymatic methods can change the nature of the protein, such as the spatial structure, the electric charge as well as the length of peptide chain. Chiou (Chiou et al., 2013) used citric acid to modify wheat gluten to produce superabsorbent materials. It was reported that a sample with a gluten: citric acid ratio of 0.5:1 and a reaction temperature of 120 °C had the largest water uptake value. Additionally, all modified gluten samples had lower thermal stability than neat gluten. The reaction of transglutaminase (TGase) with

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wheat gluten was reported to improve the physical and mechanical properties of the fiber as well as its hydrolytic stability (Jun et al., 2013).

Microwave is an electromagnetic wave whose frequency of 300 MHz–300 GHz can cause molecular vibrations at the molecular level. Microwave can also induce changes in water polarity orientation with the external electric field in high frequency, thus further causing molecular movement and mutual friction. Therefore, microwave energy converts into heat and increases the material temperature (Banik et al., 2003; Thostenson and Chou, 1999). It is reported that microwave can change the materials' structure and permanently change their properties. Accordingly, Byaruhanga (Byaruhanga et al., 2006) found that the kafirin content of  $\beta$ -folding increased after microwave treatment.

Application of microwave thermal energy is considered to be a mean for producing even heating throughout the sample as compared with direct oven heating, and thus could be used to create uniform cross-linking throughout the sample (Larhed and Hallberg, 2001). Therefore in the current study, we prepared a pure protein fiber using microwave-modified wheat protein. We investigated the effect of microwave power, time, and pH on the mechanical properties of the fiber and further optimized the process using response surface methodology. We hoped that the microwave can improve the mechanical properties of fiber and shorten the production time. Additionally, we also hoped to find the mechanism of the improved mechanical properties observed with microwave treatment.

## 2. Materials and methods

### 2.1. Materials

Commercially available wheat gluten, Whetpro 80 with about 80% protein content was purchased from Chinatex HuiZe Biotechnology (De Zhou) Co. Ltd. Analytical grade urea, sodium sulfite, sulfuric acid, sodium hydroxide, absolute alcohol, and sodium sulfate were purchased from commercial sources in China.

### 2.2. Separation of glutenin and gliadin

Wheat gluten was mixed with 65% alcohol in a solid: liquid ratio of 1:30 and stirred at 50 °C for 3 h. The mixture was then centrifuged at 4500 rpm for 20 min after the extraction. Precipitation was continued using 65% alcohol and extracted an additional 2 times. The supernatants were pooled and the alcohol was removed using a rotary evaporator at 40 °C. The gliadin-rich fraction was freeze-dried. The final precipitate was then mixed with water in a material: liquid ratio of 1:20. The pH of the mixture was regulated to pH 11.5 and stirred at 60 °C for 3 h, and then centrifuged at 4500 rpm for 20 min after the extraction. The alcohol (65%) was added to the supernatant and regulated to pH 7. The solution was then stored at 4 °C for 12 h and centrifuged (4500 rpm, 20 min). The subsequent deposits were the glutenin, which were collected and freeze-dried. The glutenin and gliadin were crushed and mixed with hexane in a solid: liquid ratio of 1:2 to remove the fat, and then passed through an 80 mesh stand-by.

### 2.3. Fiber preparation

Protein solutions with wheat gluten concentrations of 15% (w/w), and a glutenin: gliadin ratio of 1:1 at pH 7 were prepared according to the Reddy and Yang (Reddy and Yang, 2007), by dissolving wheat gluten in 8 M urea solution as a swelling agent and 1% (w/w) sodium sulfite on the total weight of the bath as the reducing agent. Wheat protein solutions were treated by

microwave under different conditions. Fibers were extruded into a coagulation bath consisting of 10% (w/w) sodium sulfate and 10% (w/w) sulfuric acid using a normal syringe and needle. The fibers formed were allowed to stay in the coagulation bath for 20 min and later rinsed with water and air-dried. Then the spun fiber was drawn and annealed. The effects of microwave power (five different levels: 100, 200, 300, 400, and 500 W), exposure time (five different times: 1, 2, 3, 4, and 5 min), and pH (four different pH: 5, 6, 7, and 8) on the breaking strength and breaking elongation of the fiber were examined.

### 2.4. Tensile testing

All of the fiber samples were conditioned before testing in a standard testing atmosphere of 21 °C and 65% relative humidity for 24 h. Fibers were tested for their tensile properties using a texture analyzer (TA). A gauge length of 40 mm and an extension speed of 2 mm/min were used for tensile testing. Approximately 15 fibers were tested for each condition and the average values were obtained.

To determine the optimal conditions for microwave technology, we chose microwave power, time, and pH as single factors. Based on this experiment, we chose the breaking strength and elongation at break as the evaluation index and determined the response surface experiment with these three factors at three levels.

### 2.5. Determination of –SH and –S–S– content in the process of wheat protein fiber preparation

The concentration of free sulfhydryl groups (–SH) of the wheat protein fiber was determined using Ellman's reagent (5/5-dithiobis(2-nitrobenzoic acid), DTNB). Changes in free sulfhydryl groups were measured in triplicate as previously reported (Beveridge et al., 1974). Briefly, finely grinded wheat protein fiber (42 mg) was diluted to 10 mL with 4.7 g guanidine hydrochloride in Tris-glycine buffer (1.04% Tris, 0.69% glycine, 0.12% EDTA (w/v), pH 8.0) and reacted for 1 h at 25 °C. A solution of 1 mL of the solution, 4 mL 8 M urea solution (dissolved with Tris-glycine buffer), and 0.1 mL Ellman's reagent was prepared and mixed in a 25 °C water bath for 30 min to avoid a light reaction. The solution was then subjected to centrifugation at 4500 rpm for 15 min, and the absorbance of the supernatant was measured at 412 nm using a UV–vis spectrophotometer.

The concentration of total sulfhydryl groups (SH) in the wheat protein fiber was measured as follows: 1 mL sample solution, 0.05 mL mercaptoethanol, 4 mL 8 M urea-5 M guanidine hydrochloride (dissolved with Tris-glycine buffer) were mixed together in a 25 °C water bath for 1 h. Then, 10 mL of 12% TCA was added and the mixture continued to stay in a 25 °C water bath for an additional 1 h. The solution was then centrifuged at 4500 rpm for 15 min. The precipitate was then washed with 5 mL 12% TCA twice. The precipitate was mixed with 10 mL 8 M urea solution and 0.04 mL Ellman's reagent in a 25 °C water bath for 30 min to avoid a light reaction. The absorbance was then measured at 412 nm using a UV-spectrophotometer. The concentration of free –SH was calculated using the following equation:

$-\text{SH} (\mu\text{M/g}) = 73.53 A_{412} \times D/C$ , where  $A_{412}$  is the absorbance at 412 nm; C is wheat protein fiber concentration (mg/mL) and D is the dilution factor. –S–S– content was calculated using the following formula:

$-\text{S}-\text{S}- (\mu\text{M/g}) = (N_2 - N_1)/2$ , where  $N_1$  is the content of sulfhydryl groups before reduction and  $N_2$  is the content of sulfhydryl groups after reduction.

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