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# Modification of protein structure and dough rheological properties of wheat flour through superheated steam treatment

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ABSTRACT

Native (NF, 13.5% w.b) and moistened (MF, 27% w.b) wheat flours were treated with superheated steam (SS) at 170 °C for 1, 2 and 4 min, and their protein structure as well as dough rheological properties were analyzed. Confocal laser scanning microscopy (CLSM) and SDS-PAGE patterns indicated the formation of protein aggregates with reduced SDS extractability after treatment. Farinograph and dynamic rheometry measurements showed that the strength as well as elastic and viscous moduli of the dough made from SS-treated flours progressively increased with SS treatment time. And both the improvements were more pronounced for superheated steam-treated moistened flours (SS-MF) than for superheated steam-treated native flours (SS-NF). Size-exclusion high performance liquid chromatography (SE-HPLC) analysis demonstrated that dough rheological parameters have positive correlations with SDS unextractable polymeric proteins (UPP) contents. SS treatment on flours led to a transition of protein secondary structures to more ordered form (α-helix and β-sheet). Additionally, free sulfhydryl (SH) contents decreased after treatment, which implied that disulfide bonds accounted for protein extractability loss and dough rheological properties improvement. Elevated moisture level promoted the modification of both protein structure and dough behaviors of flours during SS treatment.



## 1. Introduction

Wheat flour is one of the most important food materials worldwide. Huge amounts of bakery products including bread, pasta, noodles, cakes, biscuits and pastries are consumed by people every day. Gluten proteins are the principal structure forming elements of most baked foods, contributing to the elasticity, cohesiveness and viscosity characteristics of the dough. Thus, gluten proteins substantially control the quality of wheat flour based products (Misra et al., 2015). Two major protein fractions, monomeric gliadins and polymeric glutenins form the network of gluten. Inside them, gliadins interact mostly by non-covalent interaction and intrachain disulphide bonds, and glutenins consisting of high and low molecular weight subunits (HMW-GS and LMW-GS, respectively) stabilised mainly by hydrophobic interaction and interchain disulphide bonds (Lagrain et al., 2005). The glutenins have been proven to be the major components that impart strength and elasticity to the dough, while the gliadins confer viscous properties (Wang et al., 2016).

In order to improve the functionality of wheat flours several treatments have been investigated, including the use of chemical methods such as chlorination (Sinha et al., 1997), use of azodicarbonamide, KIO3, ascorbic acid (Junqueira et al., 2007), ozone treatments (Li et al., 2012), and enzymatic approaches such as use of lipoxygenase, pentosanases, proteolytic enzymes, redox enzymes (Lamsal and Faubion, 2009), and also physical treatments including high-pressure processing (McCann et al., 2013), irradiation (Azzeh and Amr, 2009) and heating, etc. Thermal treatment is more and more welcomed recently, as it is more natural and safer as well as easier applied than other processing ways. At dry heat treatment (above 50 °C), unfolding of gluten proteins occurs. The hydrophobic parts of the protein molecules are getting more exposed, which allows the rearrangement of disulfide bonds. As a

*Abbreviations:* NF, native wheat flour; MF, moistened wheat flour; SS, superheated steam; SS-NF, superheated steam-treated native flour; SS-MF, superheated steam-treated moistened flour; CLSM, confocal laser scanning microscopy; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SE-HPLC, size-exclusion high performance liquid chromatography; UPP, unextractable polymeric proteins; SH, sulfhydryl; HMW-GS, high molecular weight glutenins subunits; LMW-GS, low molecular weight glutenins subunits; FITC, Fluorescein isothiocyanate; FTIR, Fourier transform infrared spectroscopy; SD, standard deviation.

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result, gluten aggregates are forming, with decreased extractability and modified molecular weight distribution (Bucsella et al., 2016; Delcour et al., 2012). Therefore, a stronger dough or more stable foam can be produced. While at heat-moisture treatment, starch pre-gelatinization occurs and the gluten proteins suffer a nearly total loss of functional properties due to denaturation. Thus, the gluten network in the dough cannot be formed and flours with higher viscosity are produced (Bucsella et al., 2016; Delcour et al., 2012; Wang et al., 2016). However, traditional heating methods are usually time and energy consuming. Additionally, certain levels of oxidative degradation of flours will occur after long time of high temperatures processing in air environment (Wu et al., 2016).

Superheated steam (SS) processing as an emerging heating technology has recently attracted a lot of attention for its advantages. During SS processing, large amount of heat transferred to food when steam condenses on food surfaces, which rapidly increase the food temperature (Hu et al., 2016). SS processing is more efficient than saturated steam and hot air processing in heating as SS has a higher enthalpy (Hu et al., 2016). Additionally, during SS processing, foods are treated in an oxygen-free environment that can significantly reduce the oxidation loss (Wu et al., 2016). The efficiency of SS (reduced required processing time from hours and minutes to minutes or seconds) has recently been proven in several different grain processing fields, such as SS drying (Chungcharoen et al., 2015), parboiling (Soponronnarit et al., 2006), microbial decontamination (Hu et al., 2016), enzyme inactivation (Wu et al., 2014) and mycotoxin degradation (Pronyk et al., 2006). Additionally, SS technology could also be useful in the processing of other food products such as vegetables, fruits, meats and milk products, etc (Alfy et al., 2016). High efficiency, pollution free and safety make SS processing applicable in the modern food industry and it has been applied in many factories recently (Alfy et al., 2016).

Besides these processing effects, SS could also cause quality changes on the products. Products treated by SS always showed preferable properties as well as less nutritional loss than those processed by traditional heating methods (Wu et al., 2016). It was reported that SS processing (110–160 °C and 0.35–1.00 m/s for 2.5–14.0 min) significantly increased the cold paste viscosity of oat groats (Head et al., 2010). SS treatment on rice resulted in physicochemical and antioxidant properties changes (Chungcharoen et al., 2015; Rumruaytum et al., 2014). Pronyk et al. (2006) reported that high temperature SS treatment (185 °C and 1.3 m/s for 4 min) on wheat kernels caused partial gelatinization of starch, which was beneficial for alcohol production and animal feeding. However, effects of SS, which has a much higher temperature than conventional heating (50–100 °C), on the protein structure as well as dough rheological properties of wheat flour rarely reported.

Flour with increased moisture content showed a higher mobility of the molecules during heat treatment and thus enhanced the alterations in structure and functional properties (Mann et al., 2013). In this study, both native (NF, 13.5% moisture content) and moistened (MF, 27.0% moisture content) wheat flours were subjected to SS treatment at 170 °C for 1, 2 and 4 min. Effects of SS processing on microstructure, molecular weight distribution, SDS extractability, free sulfhydryl (SH) content and secondary structure of proteins were investigated. In addition, alterations in dough properties of flours were also analyzed by using both fundamental (dynamic oscillation) and empirical (farinograph) rheological measurements.

## 2. Materials and methods

### 2.1. Materials

Soft wheat flour (13.5% moisture content, 8.5% protein) was kindly provided by Xinliang grain processing Corporation (Henan, China). All flours were stored at 4 °C before using. To achieve flour with moisture content of 27.0%, flour sample was placed in a wide tray. Subsequently, the flour was hydrated with the required volume of demonized water applied with a spray bottle. Flour-water mixture was then transferred into a polyethylene bag and vacuum sealed. The sealed sample was equilibrated for 24 h at 4 °C.

Fluorescein isothiocyanate (FITC) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Rhodamin B was purchased from Kermel Chemical Reagent Co. Ltd. (Kermel, Tianjing, China). All the chemical reagents were analytical grade.

### 2.2. Superheated steam processing

A SS processing system developed by Laboratory of Cereal Science at China Agricultural University was used in this study. The schematic diagram was detailed showed in our previous report (Hu et al., 2016). Processing of NF and MF samples was conducted at atmospheric pressure in the SS chamber at 170 °C for 1, 2 and 4 min. The steam velocity was 15.0 $m^3$/h. When the SS temperature was stable at 170 °C, wheat flour (200 g) was scattered on the sample tray and conveyed into the processing chamber using the conveyor belt. After treated for the set time, flour sample was conveyed out of the processing chamber and then cooled at room temperature. The treated flour samples were freeze-dried and re-ground to powder with a grinding mill (Huanyatianyuan, HY-04A, China), subsequently, they were passed through a 100-mesh sieve. Moisture contents of finally flour samples were 6.1%–8.3%. The same water basis (14%, w.b) was used for all the flour samples in analytical and rheological experiments below to avoid the differences originated from different moisture contents of flours. Untreated NF was used as the control sample. All the samples were stored at 4 °C for further analysis. The SS processing of wheat flours was performed in duplicate.

### 2.3. Confocal laser scanning microscopy (CLSM)

CLSM is a valuable method for a deeper understanding of the microstructure of the proteins and starches in flour owing to its ability to select and differentiate particular structures in the food system, with the application of staining procedures. A solution of Fluorescein isothiocyanate (FITC, 1 g/ml) and Rhodamin B (0.1 g/ml) was used for non-covalent labeling of starch (green) and proteins (red), respectively. To prepare stained flour sample, firstly, 40 mg of flour was dispersed in 1 ml of distilled water. Then, the flour suspension was thoroughly mixed with a 100 μL aliquot of the FITC and Rhodamin B solution. Subsequently, mixtures were kept in the dark at room temperature for 1 h. Then, a small amount of stained flour sample was placed on a concave slide. Following by, a glycerol-coated cover slip was covered on it. The observation of microstructure of flour samples was applied using a confocal scanning laser microscope (Leica, TCS SP5, USA) in ×40 objective. CLSM images, acquired in 512 × 512 pixel resolution, were analyzed using a ZEN2012 software. The excitation wavelengths for FITC and Rhodamin B were 488 and 543 nm, respectively. The ranges of fluorescence emission of FITC and Rhodamin B for starch and protein were 450–540 nm and 545–660 nm, respectively.

### 2.4. Size-exclusion high performance liquid chromatography (SE-HPLC)

Protein SDS-extractability of flours at different treatments were determined using SE-HPLC (1260, Agilent, USA) according to the method reported by Luo et al. (2016) with some modifications. Firstly, each flour samples with calculated qualities (has same dry