



In vitro digestion and physicochemical properties of wheat starch/flour modified by heat-moisture treatment



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ABSTRACT

Physicochemical and digestive properties between wheat starch (WS) and wheat flour (WF) which had been modified by using heat-moisture treatment (HMT) were investigated. Reduced peak viscosity and increased pasting temperature were found in both WS and WF after HMT. Samples treated with a higher moisture treatment (25% and 35%) exhibited biphasic endotherms. HMT significantly changed crystal structure of WS and WF, and patterns transferred from A to A + V with moisture content increase indicating a formation of starch–lipid complex. Besides, HMT caused the clumping of starch granules and the aggregation of denatured protein, observed using confocal laser scanning microscopy (CLSM) and light microscopy. The higher moisture contents produced HMT samples with higher resistant starch content. In addition, HMT wheat flour showed a higher resistant and slowly digestible starch content when compared to the other counterpart of WS.

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1. Introduction

Heat-moisture treatment (HMT) of flours allows control of molecular mobility at higher temperatures by limiting the amount of water. HMT promotes interaction of polymer chains by disrupting the crystalline structure and dissociating the double helical structure. This is followed by rearrangement of the disrupted crystals (Gunaratne and Hoover, 2002). This influences granular swelling, solubility, pasting properties, granule morphology and susceptibility to enzymatic or acidic hydrolysis (Zavareze and Dias, 2011). Many researches have studied the HMT effect on the physicochemical properties of different starch species (Adebowale et al., 2009; Chung et al., 2009; Gunaratne and Hoover, 2002; Jiranuntakul et al., 2011). Studies comparing the properties of flour and starch modified by HMT seem to be limited. Blazek and Copeland (2008) investigated the influence of the contents of

total amylose, free and lipid-complexed amylose, and amylopectin chain length distribution on swelling behavior and pasting properties of wheat flour and starch with different amylose content. Rice and sorghum flour/starches have been modified by HMT recently (Puncha-arnon et al., 2013; Sun et al., 2014), showing that HMT has a greater impact on the paste viscosity and thermal properties of sorghum and rice flour than starch. Components in rice flour other than rice starch granules underwent alteration during HMT with proteins playing an important role in change of properties in the modified rice flour samples (Puncha-arnon and Uttapap, 2013).

The glycemic index (GI) has been transformed from a potentially useful tool for planning diabetic patient diets to promising strategies for the prevention of diabetes, dyslipidemia, cardiovascular disease, and even certain cancers in the general population (Jenkins et al., 1981). Clinical data have shown that a low GI diet is linked with reduced risk of diabetes and cardiovascular disease (Jenkins et al., 2002). Rapidly digestible starch (RDS) is thought to promote a stronger metabolic syndrome, which would influence insulin resistance, obesity, and diabetes. The benefit of slowly digestible starch (SDS) is the moderate impact on the GI. Resistant starch (RS) within a calorie-controlled diet is thought to be beneficial in protecting against metabolic syndrome and colon cancer (Jenkins et al., 2002).

Effects of HMT on the digestibility of potato starch (Lee et al., 2012), corn, pea, and lentil starches (Chung et al., 2009), mung

Abbreviations: BD, breakdown; BU, Brabender units; Cv, cold paste viscosity; DSC, differential scanning calorimetry; GI, glycemic index; GOPOD, glucose oxidase/ peroxidase; ΔH , enthalpy; HMT, heat moisture treatment; Hv, hot past viscosity; LSD, least significant difference; NWF, native wheat flour; NWS, native wheat starch; Pv, peak viscosity; RDS, rapidly digestible starch; RS, resistant starch; SB, setback; SDS, slowly digestible starch; T_c , conclusion temperature; T_o , onset temperature; T_p , pasting temperature; T_p , peak temperature; WF, wheat flour; WS, wheat starch; XRD, X-ray diffraction.

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bean starch (Li et al., 2011) and canna starch (Juansang et al., 2012) have been reported. Results showed that temperature, moisture content and the reaction time of HMT have an influence on starch digestibility. For example, *in vitro* digestion and physicochemical properties of A- and B- granules separation from soft and hard wheat flours have been investigated, showing that the protein content, amylose and amylopectin fine structure, proportion of A- and B- wheat starch granules and granular size could be the major factors that affect the digestion and other functional properties (Liu et al., 2007). Lerdluksamee et al. (2013) compared starch digestibility of *Scirpus grossus* flour and starches, finding that *Scirpus grossus* starch had a higher digestion rate than those of the flours. Besides, Zhang and Hamaker (1998) indicated that protein in cooked sorghum flour pastes play an important role in making slowly digestible starch. After the flours were predigested with pepsin to remove some proteins, the starch digestibility of cooked sorghum flours increased 7–14%.

Wheat flour provides the basis for breads and other baked products including noodles, cookies and snacks. To broaden the application in functional foods with good nutritional properties, it is necessary to modify wheat flour. Wheat flour consists of starch granules and non-starch components including non-starch polysaccharides, lipids and proteins. The interactions between starch and non-starch components of flour during HMT are possibly different from that of starch.

Little work has been reported on the *in vitro* digestion differences between wheat starch and wheat flour modification by HMT. This study investigates the structural and functional properties of wheat starch/flour modified by heat-moisture treatment. The light microscopy, pasting, thermal and crystallization properties along with *in vitro* digestibility of wheat starch and wheat flour after HMT were observed. Due to the popularity and need of novel market foods with good nutritional properties, there may be a potential application in the functional noodle industry in adding modified wheat flour for positive health effects.

2. Materials and methods

2.1. Materials

Wheat flour was obtained from Luwang Company (Shandong, China). Pancreatin from porcine pancreas (Cat. No. P7545, activity 8 × USP) and amyloglucosidase (Cat. No. A7095, activity 300 unit/mL) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Glucose oxidase-peroxidase (GOPOD) assay kits were purchased from Megazyme International, Ltd. (Co. Wicklow, Ireland). Other chemicals used in the study were all analytical grade.

2.2. Wheat starch preparations

Wheat starch was isolated from wheat flour by using a modified batter procedure (Massaux et al., 2008). Wheat flour (2.0 kg) was mixed with 60% (flour hydration capacity basis) water for 2 min. The resulting dough was then allowed to rest for 8 min. 2.0 L of water was then added, and the dough was stirred for 25 min. The mixture was transferred into a vessel with 10.0 L of water. The diluted suspension was then stirred continuously for 35 min to agglomerate the gluten. 10.0 L of water and the mixture were then pumped over vibrating sieves with decreasing pore size (400, 250, 125, 90 and 50 μm). The sieves retained gluten, fibers and starch in the filtrate. The starch suspension was allowed to rest for 24 h at 4 °C following which the supernatant was decanted. Centrifugation was then used again for 10 min at 3000 g and the supernatant was decanted again. The sediment consisted of a yellow-brown layer of

sludge fraction with a starch layer underneath. The top layer was scraped off and the white bottom layer was re-suspended in water. This suspension was then centrifuged a second time with the top layer again scraped off, re-suspending the starch layer in water and centrifuging a third time. About 1.0 kg starch was collected, and then dried in an oven at 40 °C for 24 h. The dried starch was ground in a blender and then passed through a 100 μm sieve.

2.3. Chemical composition of wheat starch and flour

Standard AOAC methods (2000) were used for the measurement of moisture, protein, ash and lipid. Protein was determined by estimating the total nitrogen using a conversion factor of 6.25. Apparent amylose content was determined by a procedure described by Yoo and Jane (2002). Total starch was then determined using a total starch assay kit (K-TSTA 07/11, Megazyme International, Wicklow, Ireland).

2.4. Heat-moisture treatment

Samples (wheat starch and wheat flour) were weighed in a glass container, and the moisture content was adjusted to 15%, 25% and 35% and equilibrated for 24 h at room temperature. The moisture content was measured using a moisture analyzer (MA-45, Sartorius AG, Goettingen, Germany). The mixture was placed in sealed containers and kept for 24 h at 120 °C in a convection oven. After cooling to room temperature, the samples were dried at 40 °C overnight and then passed through 100-mesh sieve for further analysis. Untreated wheat starch and flour were then used as controls.

2.5. Pasting properties

Samples dispersions (6%, w/w, db) were made with a certain ratio of sample and water and then analyzed using a Micro Visco-Amylo-Graph (Brabender, Germany) following the method reported by Chang et al. (2013). The pasting temperature and peak, breakdown, and setback viscosity were recorded.

2.6. Light microscopy

Light micrographs were performed using an Olympus BX-51 light microscope (Tokyo, Japan) with brightfield and polarized light. Sample powder was sprinkled on a glass slide and covered with a single drop of an aqueous glycerol solution (1:1 water/glycerol). The dispersed samples were covered with a coverslip. Images were recorded at 500 × magnification.

2.7. Confocal laser scanning microscopy (CLSM)

The CLSM-images of mixtures were recorded at room temperature with a Leica TCS SP5 Confocal Laser Scanning Microscope (Leica Microsystems Inc., Heidelberg, Germany), equipped with an inverted microscope (Model Leica DMI6000).

Nile Blue was used to stain the starch particles. A 0.01 wt.% Nile Blue staining solution was prepared by mixing 0.001 g of Nile Blue dye in 10 mL of MilliQ water at room temperature. The solution was stored in a dark place. Nile Red was used to stain the protein phase. A 0.01 wt.% Nile Red staining solution was prepared by dissolving 0.001 g of Nile Red dye in 10 mL of 1,2-propanediol. The solution was stored in a dark place. All these solutions were prepared at room temperature (20 ± 3 °C). Approximately 10 mg samples were thoroughly mixed with a 10 μL aliquot of the Nile Red and Nile Blue solutions. Then the stained samples were stored at ambient temperature for at least 1 h before observation. A small amount of stained mixture was placed on a concave slide, which was then

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