PREPARATION, CHARACTERIZATION, AND EVALUATION OF SORGHUM FLOUR WITH INCREASED RESISTANT STARCH CONTENT

by

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Abstract

Sorghum flour is a gluten-free ingredient and can be used to prepare foods for celiac patients. In addition, sorghum flour is a good source of fiber in the form of resistant starch. The objectives of this research were to develop an effective process to increase resistant starch content of sorghum flour and investigate the effects of sorghum protein matrix on starch digestibility. Samples of white sorghum flour (28.9% amylose content) with different moisture contents (0%, 12.5%, 20%, and 30%) were treated at different temperatures (100, 120, and 140^{o}C) for different times (1, 2, and 4 h). Samples after heat treatments were tested for starch digestibility, protein digestibility, differential scanning calorimetry (DSC), size-exclusion chromatography (SEC), and X-ray diffraction. The sample treated with 20% moisture at 100^{o}C for 4 h had high resistant starch (RS) content (22.1% compared with 5.6% of the native sample) and low protein digestibility (8.4% compared with 68.3% of the native sample). The same heat-moisture treatment on isolated sorghum starch showed no significant change in RS content. DSC showed a very low degree of gelatinization for samples treated at moisture contents 20% and below. X-ray diffraction also suggested minimal change in starch crystallinity after heat treatment at low moisture contents (20% and below). Sorghum protein solubility after heat treatment was reduced, suggesting that protein structure was altered during the heat treatments. In conclusion, heat-moisture treatments were successful in increasing resistant starch content of sorghum flour by altering sorghum protein without gelatinizing the starch to retain starch functionality in food product applications. Sorghum flour with increased resistant starch content after heat treatment was evaluated and compared with normal sorghum flour for starch digestibility using the Integrated Total Dietary Fiber method, and for food applications in tortillas.

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Dedication

To my parents for your unconditional love...

Chapter 1 - Preparation and characterization of sorghum flour with high resistant starch content

Introduction

Starch is classified based on the rate of digestion as rapid digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RS is the portion of the starch that resists enzymatic digestion in the small intestine (Englyst et al., 1992; Sajilata, Singhal, & Kulkarni, 2006) and can be classified into five different types: RS1 is resistant because of physical barriers that prevent enzymes from accessing and digesting starch; RS2 is granular resistant starch that cannot be digested completely due to its granular structure; RS3 is retrograded starch formed after cooking and cooling in which the amylose is re-arranged into a crystal form and is not digestible; RS4 is chemically modified starch such as cross-linked starch; and RS5 is a new RS category based on amylose-lipid complex (Hasjim et al., 2010). Because RS can escape digestive enzymes in the small intestine, RS can positively affect blood glucose (Behall, Scholfield, Hallfrisch, & Liljeberg-Elmstahl, 2006) and insulin levels (Behall et al., 2006; Maki et al., 2012), and improve colonic health by increasing fecal bulk and short chain fatty acid (SCFA) (Jenkins et al., 1998). Experiments in which rats consumed RS have shown its potential for reducing body fat (Keenan et al., 2006) and preventing colonic cancer (Bauer-Marinovic, Florian, Müller-Schmehl, Glatt, & Jacobasch, 2006). Other health benefits of RS are promoting the growth of beneficial microorganisms in the large intestine, reducing the formation of gall stones, inhibiting fat accumulation, and increasing the absorption of important minerals such as calcium and iron (Sajilata et al., 2006). With many benefits of resistant starch, increasing resistant starch content in foods would be beneficial, especially in the United States where obesity and diabetes are prevalent.

Different methods have been used to improve RS content of cereal starches and flours including chemical and thermal treatments. Chemical modification has been used to create starches with high levels of RS such as citrate starch (Wepner, Berghofer, Miesenberger, Tiefenbacher, & NK Ng, 1999; Xie & Liu, 2004), and cross-linked wheat starch (Woo & Seib, 2002). Pyrodextrinization and crosslinking were used successfully to increase RS content of the African locust bean (Sankhon et al., 2013). Thermal treatments, including heat-moisture treatment, also have been used successfully to increase RS content of different starches. Unlike

annealing treatment, which uses excess water and low temperature, heat-moisture treatment requires high temperature and low moisture content (less than 30%). Both annealing and heat-moisture treatment are methods of modifying starches with minimum occurrence of starch gelatinization (Zavareze & Dias, 2011). Heat-moisture treatments has been reported to affect the physicochemical properties and functionality of different starches including sorghum (Olayinka, Adebowale, & Olu-Owolabi, 2008; Singh, Chang, Lin, Singh, & Singh, 2011; Sun, Han, Wang, & Xiong, 2014), normal and waxy potato (Jiranuntakul, Puttanlek, Rungsardthong, Puncha-Arnon, & Uttapap, 2011; Varatharajan, Hoover, Liu, & Seetharaman, 2010; Kim & Huber, 2013), normal and waxy rice, normal and waxy corn (Jiranuntakul et al., 2011), and millet (Amadou, Gounga, Shi, & Le, 2014). Both decreased and increased susceptibility to α-amylase of different starches after heat-moisture treatments were reported by Hoover & Vasanthan (1994). Heat-moisture treatment with low moisture content (<30%) and high temperature (80-140 ^{o}C) has been shown to improve SDS and RS content of corn, pea, and lentil starches (Chung, Liu, & Hoover, 2009), increase SDS content of sweet potato (Shin, Kim, Ha, Lee, & Moon, 2005), normal and waxy corn kernels (Wongsagonsup, Varavinit, & BeMiller, 2008), cocoyam, yam, plantain and rice flours (Niba, 2003), and increase RS content of high-amylose corn starch (Shi & Trzasko, 1997), and African locust bean starch (Sankhon et al., 2013). Heat-moisture treatment was used in combination with chemical and enzymatic modification to manipulate starch digestibility. The combination of acidic conditions and heat-moisture treatment on corn starch resulted in higher level of boiling-stable RS when applied to normal and high-amylose corn starches (Brumovsky & Thompson, 2001; Lin, Singh, Wen, & Chang, 2011). Heat-moisture treatment combined with mildly acidic conditions (pH 5 to 6.5) was shown to increase levels of thermally-stable RS content of potato starch (Kim & Huber, 2013). A combination of heat-moisture treatments and phosphorylation on high-amylose corn starch was shown to result in high RS content that can survive cooking (Sang & Seib, 2006). Enzymatic modification combined with hydrothermal treatments can be used in the formation of RS in red kidney bean starch (Reddy, Suriya, & Haripriya, 2013). In summary, different methods can be used to increase RS content of starches, but the process usually involves adding chemical groups to the starch, or using specialty materials (e.g. high amylose cornstarch).

Sorghum flour is an excellent material for heat-moisture treatment to obtain high resistant starch content. Sorghum is a staple food in semi-arid areas of the world, but most of the sorghum

production in the U.S. is for feed and biofuels, with only a small portion used for food (Murty & Kumar, 1995). Compared with corn starch, sorghum starch in flour is known to be less digestible (Elkin et al., 2002; Rooney & Pflugfelder, 1986). In sorghum flour, protein exists mostly on the outside of starch granules, forming a physical barrier on the surface of starch granules, and making the starch granules inaccessible for enzymatic digestion. This could be an advantage of sorghum flour in preparing RS. Studies also show that after cooking sorghum and corn flour, sorghum protein digestibility decreases, whereas corn protein digestibility does not (Hamaker, Kirleis, Mertz, & Axtell, 1986; Zhang & Hamaker, 2003). The decrease in sorghum protein digestibility after cooking is attributed to increases in disulfide cross-links of sorghum protein β and γ kafirins (Duodu, Nunes, Delgadillo, Parker, Mills, Belton, & Taylor, 2002), that help strengthen the protein-matrix barrier around starch granules. Thus, thermal treatment may increase the RS content of sorghum flour (which is higher in RS content than corn) without gelatinizing the starch to preserve native starch functionality in food applications. Heat- moisture treatment has been used to modify physicochemical properties and functionality of sorghum starches (Adebowalea, Olu-Owolabi, Olayinka, & Lawal, 2005; Olayinka et al., 2008; Singh et al., 2011; Sun et al., 2014); however, information is limited on the effect(s) of heat-moisture treatment on the digestibility of starch and protein in sorghum flour, where the heat-moisture treatment can change protein properties and consequently affect starch digestibility with minimal change in starch properties and functionality. The objectives of this study were to develop a hydrothermal treatment to increase RS content of sorghum flour without gelatinizing the starch, to investigate the effect of treatment conditions on sorghum protein matrix, and to understand how the protein matrix affects starch digestibility.

Materials and methods

Materials

Food-grade white sorghum flour was obtained from Archer Daniels Midland Company (Decatur, IL). Food-grade white sorghum flour was obtained from Archer Daniels Midland Company (Decatur, IL). Test kits, including starch damage, total starch, and amylose/amylopectin, were purchased from Megazyme (Megazyme International Ireland, Co., Wicklow, Ireland). Commercial protease enzyme (Protex 6L) was obtained from Genecor International (Palo Alto, CA). Pepsin, pancreatic α-amylase, and amyloglucosidase were purchased from Signma Aldrich (St. Louis, MO).

Methods

Sorghum flour composition

The sorghum flour was tested for damaged starch content using AACI Method 76-30.02, total starch content using AACC Method 73-13.01, and amylose/amylopectin content using the Concanavalin A method (Gibson, Solah, & McCleary, 1997). Moisture content was determined using the AACC Air Oven Method 44-19. Protein content was obtained by nitrogen combustion using a LECO system (LECO Corporation, St. Joseph, MI). Resistant starch content of the flour was determined followed Englyst et al. (1992)

Heat treatments of sorghum flour

Dry heat treatments

Sorghum flour (25g) was spread on aluminum foil and dried at 100^{o}C for 30 min. The flour was then heated at different temperatures (100^{o}C, 120^{o}C, and 140^{o}C) for different times (1, 2 and 4 h). After heating, samples were cooled and left overnight on the bench top for moisture equilibration.

Heat-moisture treatments

Appropriate amounts of distilled water were added to 25 g of sorghum flour to adjust moisture content (wet basis) to 20% and 30% and the samples were whisked to thoroughly distribute the water into the flour. Sorghum flour with moisture content of 12.5%, 20% or 30%

was sealed in canning jars and heated for 1, 2, and 4 h at 100^{o}C. For heat treatments at 120^{o}C, and 140^{o}C, flour samples were sealed in pressure bottles (250-ml bottles, Ace Glass Incorporated, Vineland, NJ) to prevent moisture loss and heated for 1, 2 and 4 h. After heat-moisture treatment, flour samples were transferred to Petri dishes for drying and moisture equilibration overnight at room temperature.

Starch isolation and heat treatments of sorghum starch

Sorghum starch isolation

Sorghum flour (125g) was suspended in distilled water (400ml) and pH of the suspension was adjusted to 10.0 using 0.25M sodium hydroxide. One ml of commercial protease enzyme (Protex 6L, Genecor International, Palo Alto, CA) with enzyme activity of 580,000 DU/g (with 1000 DU means 1ml of 2% enzyme solution provides a difference of extinction of 0.400 at assay conditions of casein, pH 8.5, 40^{o}C) was added, and the suspension was incubated at 40^{o}C for 18 h with continuous stirring using a magnetic stir bar. After incubation, the mixture was passed through a 200 mesh sieve and contents that passed through were centrifuged at 2,000 g for 15 min. The top layer (tailings portion) of the pellet was scraped away and discarded. The sediment containing prime starch was re-suspended in water, mixed, and centrifuged at 2,000 g for 15 min. The process was repeated twice, until the all tailing material was completely removed. The final pellet was oven-dried at 40^{o}C for 48 h.

Heat treatments of sorghum starch

Similar to sorghum flour, sorghum starch was heated for 4 h at 100^{o}C, 120^{o}C, and 140^{o}C with moisture contents of 0%, 9.8 % (as-is), 20%, and 30%. Samples after heat treatments were cooled, dried (if needed), and left on the bench top overnight for moisture equilibration.

Samples of sorghum starch before and after heat treatments were analyzed for starch digestibility, thermal properties, and crystallinity.

Starch digestibility

Starch digestibility was measured by a modified *in vitro* Englyst method (Englyst et al, 1992). Briefly, flour samples (0.6 g) and guar gum (50 mg) were placed in a centrifuge tube (45 ml). Freshly prepared pepsin solution (50 mg pepsin in 10 ml 0.01M HCl) was added to the tube, and the mixture was incubated for 2 h at 37^{o}C. Sodium acetate solution (0.25M) was added to the

mixture to stop digestion. Pancreatic/amyloglucosidase mixture (5 ml) and glass beads were added to the sample tube for starch digestion. The tube was incubated in a shaking water bath at 37^{o}C and 180 strokes/min. At 20 and 120 min, a 0.25 ml aliquot of sample was pipetted into 10 ml of 66% ethanol. The glucose released at each interval was determined using the glucose oxidase/peroxidase method and was converted to a percentage of starch hydrolyzed by multiplying by 0.9. Starch digested at 20 min was defined as RDS, starch digested between 20 and 120 min was defined as SDS, and starch not digested after 120 min incubation was defined as RS.

Light microscopy

Sorghum flour and heat treated sorghum flour samples were dispersed in 50% glycerol solution at a flour concentration of 10mg/ml. A drop of sample suspension (~20µl) was deposited on a glass microscope slide, covered with a cover slip, and viewed with an Olympus BX 51 microscope (Olympus America Inc., Melville, NY) using a 40x objective under bright field and polarized light. Images were captured using SPOT 4.6 Windows software (Diagnostic Instrument Inc., Sterling Heights, MI).

Differential Scanning Calorimetry of flour and starch samples

Each sorghum flour or starch was combined with distilled water in a DSC pan to achieve a ratio of 1:2 of sample dry weight to water. The pan was sealed and analyzed with an empty pan used as reference. A TA Q200 system (TA Instruments, New Castle, DE) was used to obtain the heat flow curves from 10 to 140^{o}C at a heating rate of 10^{o}C/min. The onset (To), peak (Tp), and conclusion (Tc) temperatures, and enthalpy (ΔH) associated with gelatinization of starch was obtained by analyzing heat flow curves using QA Universal Analysis software (TA Instruments, New Castle, DE).

X-ray diffraction

Samples of sorghum flour and sorghum starch before and after heat treatments (4h treatments) were adjusted to 18 % moisture content in a sealed dessicator with 100% humidity at room temperature for 2 days. Diffraction diagrams were recorded using X-ray diffraction system (Rigaku MiniFlex II) operated at 35kV and 30mA in the Debye-Scherrer transmission mode with

X-ray radiation Cu-Kα (λ=0.15405 nm). X-ray diffractograms were obtained between 3° and 35° (θ) at 0.05° increments.

Protein digestibility

Protein digestibility of sorghum flour before and after heat-moisture treatment was measured using a modified protein digestibility assay (Mertz et al., 1984). Flour samples (200mg/sample) were weighed and placed in 50ml centrifuge tubes. Each sample was incubated with 35ml pepsin solution (1.5 mg pepsin in 1ml of 0.1 M phosphate buffer pH2.0) at 37^{o}C. After 2 h of incubation, 2ml of 2M sodium hydroxide was added to each tube to stop the digestion. All tubes were centrifuged at 3320g for 15 min at 4^{o}C and the supernatant was discarded. The residue was washed in 10 ml of 0.1 M phosphate buffer pH 2.0, centrifuged, and supernatant was discarded. The washing steps were repeated one more time, and samples were frozen (-80^{o}C for 30 min) and lyophilized. Freeze-dried samples were subjected to nitrogen combustion (LECO system) to analyze the amount of undigested protein. Digested protein was calculated based on protein content of the native sorghum flour and that of the undigested fraction.

Protein extractability and size-exclusion high-performance liquid chromatography (SEC)

Protein extraction and SEC were carried out following the method described by Ioerger et al. (2007) with minor modifications. One ml of 12.5mM sodium borate buffer pH 10 with 2% sodium dodecyl sulphate (SDS) was added to 20 mg of sorghum flour in 2ml centrifuge tubes. Samples were mixed using a Fisher Genie 2 vortex mixer for 30 min. After mixing, samples were centrifuged for 4 min at 4000g and supernatants were transferred to clean-2ml tubes, and heated at 80^{o}C for 2 min to deactivate any proteases. After heat deactivation, samples were transferred to HPLC vials for analysis of the non- reduced protein fraction. The remaining pellets after centrifugation were extracted again with 1ml of 12.5mM sodium borate buffer pH 10 with 2% SDS and 2% β-mercaptoethanol. The samples were mixed by vortex mixer for 30 min and centrifuged (4000g for 4 min). The supernatant was collected, heat deactivated, and transferred to HPLC for analysis of the reduced protein fraction.

SEC analysis of non-reduced and reduced protein fraction was performed using an Agilent 1100 HPLC system and a Biosep S-3000 column (Phenomenex, Torrance, CA), with 50mM sodium phosphate at pH 7.0, with 1% SDS used as the mobile phase at a flow rate of

1ml/min. HPLC peak areas of non-reduced and reduced fractions were obtained using Agilent Chemstation software. The combined area of non-reduced and reduced fractions was the total HPLC area of sample. Protein extractability was calculated as % protein extractability=100%*total area of sample/total area of control.

Protein surface hydrophobicity test

Sorghum flour samples with different heat treatments were subjected to protein hydrophobicity test using the bromophenol blue (BPB) method (Chelh, Gatellier, & Santé-Lhoutellier, 2006). Flour (50 mg) was weighed in 2 ml centrifuge tubes. One ml of 20mM sodium phosphate buffer (pH 6.0) was added to the tube followed by 200µl of 1mg/ml BPB in de-ionized water. The tubes were vortexed on a 30-tube-holder vortex mixer for 10 min. After vortexing, sample tubes were centrifuged for 15 min at 2000 g. Supernatant (100µl) of each sample was transferred to a new centrifuge tube and 900µl of the 20mM sodium phosphate buffer was added. The diluted samples were mixed and read at 595 nm against a blank (sodium phosphate buffer) on a UV spectrophotometer. The amount of BPB bound representing the protein surface hydrophobicity of samples was calculated as: BPB bound (µg) = 200µg (Abs of control samples – Abs of sample)/Abs control.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of sorghum flour samples with different heat treatments were collected with a Perkin Elmer Spectrum 400 FT-IR/FT-NIR spectrophotometer in the frequency range of 4000 cm^{-1} to 400 cm^{-1}. Flour samples were added into the sample holder and scanned (32 scans at 4 cm^{-1} resolution).

Statistical analysis

Experiments on sorghum flour before and after heat treatments were done at least in duplicate. Collected data were analyzed by analysis of variance (ANOVA) with Duncan's multiple range test using SAS version 9.2 (SAS Institute Inc., Cary, NC). Mean values and standard deviations were reported.

Results and Discussion

Sorghum flour

The flour as received had a damaged starch content of 10.7%, moisture content of 12.5% (wet basis), protein content of 10.0% (dry basis), starch content of 86.2% (dry basis), and RS content 5.6% (starch basis). The flour was normal sorghum flour with amylose content of 28.9 % (starch basis)

Starch digestibility

Sorghum flour

RDS, SDS and RS contents of sorghum flour before and after heat treatment are shown in Figure 1.1. Increases in RDS contents after heat treatment were observed for heat treatment with 30% moisture content (MC) at all temperatures and heat treatments with 20% MC at 140^{o}C (Figure 1.1.a). RDS contents of flour treated with lower MC (0% and 12.5%) at all temperatures and 20% MC at 100^{o}C and 140^{o}C remained similar to that of the native flour. At higher temperature and MC, starch gelatinization occurred, making the starch more digestible and resulting in increased RDS levels. Further testing using light microscope and DSC would confirm the degree of gelatinization of heat treated sorghum flour.

Figure 1.1.b shows decreases in SDS levels as the RDS content increased for samples treated at 30% MC and 20% MC at 120^{o}C, and as RS content increased for samples treated at 20% MC at 100^{o}C and 120^{o}C, and 12.5 % MC at 140^{o}C for 4 h. Although other studies have shown increases in SDS content of sweet potato (Shin et al., 2005), pea, corn, lentil (Chung et al., 2009), and cocoyam, plantain, and rice (Niba, 2003), heat treatments affected SDS content of sorghum flour in this study differently. In sorghum flour, SDS content decreased after heat treatments, and decrease in SDS was replaced by RDS or RS content depending on the heat treatment conditions.

While heat-moisture treatments increased RDS and decreased SDS, RS levels were increased (Figure 1.1.c). Samples of sorghum flour with 20% MC heated for 4 h at 100^{o}C and 120^{o}C and samples with 12.5% MC heated at 140^{o}C for 4 h were found to have a significantly higher RS content (22.1%, 18.7%, and 24.5%, respectively) compared with RS content of

untreated sorghum flour (5.6%). The increase in RS content of heat-treated sorghum flour was larger than that previously reported by (Chung et al., 2009) for corn, pea, and lentil starches. Thermo-stable RS contents increase after heat-moisture treatment of potato starch under mildly acidic pH, where amylopectin molecules are hydrolyzed under acidic conditions and re-associate to become resistant to enzyme digestion (Kim & Huber, 2013). Heat-moisture treatment alone (e.g. without pH adjustment or enzymatic pretreatment) is not very effective at increasing RS content of other starches compared with sorghum flour.

Sorghum starch

RDS, SDS, and RS contents of native sorghum starch and sorghum starch that was heat-treated for 4 h are shown in Figure 1.2. RDS content of isolated starch increased slightly with the high moisture and temperature of heat treatments (Figure 1.2a), which is similar to results of heat treatments on sorghum flour. SDS levels decreased slightly with increased moisture of the heat treatments (Figure 1.2 b). RS content increased for samples heated at 100^{o}C and 20% MC (Figure 1.2c), but the increase was not statistically significant. With the majority of the protein removed during the isolation process, isolated sorghum starch did not show a significant increase in RS content compared with the same heat treatment on sorghum flour. Thus, the change in RS content of heat treated sorghum flour cannot be explained only by changes in starch properties. RS contents of native sorghum flour and sorghum starch, as well as heat treated sorghum flour and sorghum starch at 100^{o}C, 4h, and 20% MC, are shown in Table 1.1. Interestingly, isolated starch from heat treated sorghum flour had a high RS content.

Changes in sorghum starch after heat treatments

Morphology and birefringence

Figures 1.3, 1.4, and 1.5 show the changes in the birefringence of heat-treated sorghum flour. Samples treated for 4 h at 120^{o}C and 30% moisture, and at 140^{o}C with 20% or 30% moisture had major birefringence losses (Figure 1.4 and 1.5). These results indicate a higher degree of gelatinization for samples treated at higher temperatures with high MC; they also explain the increase in starch digestibility and RDS content of sorghum flour treated at high temperature and high MC (Fig 1.1.a), where the starch granule structures were disassociated and

become accessible for enzyme digestion. Differential scanning calorimetry and x-Ray diffraction on these samples were used to further differentiate the degree of gelatinization.

Thermal properties

Gelatinization properties

Figure 1.6 and Table 1.2 show the changes in thermal properties of sorghum flour after different heat treatments. Native sorghum flour was characterized by an endothermic peak at 73.1^{o}C with enthalpy (ΔH) of 7.4 J/g. Dry heat treatment had no effect on the endothermic peak and ΔH because no water was present to facilitate molecular mobility even at 140^{o}C. As MC and temperature of heat treatment increased, decreases in ΔH were observed with the most pronounced effect at 30% MC and 140^{o}C. The endothermic peak of gelatinization shifted to a a higher temperature and narrowed with heat treatment of 20% MC at 100 and 120^{o}C, and 12.5% MC at 140^{o}C (Figure 1.6). The results indicated melting of weak starch crystals and growth and perfection of existing crystals during heat-moisture treatment under high temperature. Sorghum flour treated with 30% MC at all temperatures and 20% MC at 140^{o}C had a broadened endothermic peak compared with the unmodified flour (Figure 1.6). At high MC, enough water was present to facilitate molecular mobility at the higher temperatures and disrupt crystalline structures, leaving only a small amount of the stronger crystals that appeared in the DSC thermogram as broad peaks at higher temperature compared with the endothermic peak of native sorghum flour. There were also changes in the thermal properties of the starch in flour samples treated with 20% MC at 100^{o}C and 120^{o}C, and with 12.5% MC at 140^{o}C, but the changes were not significant enough to explain the increase in RS content of heat treated sorghum flour under these conditions.

Enthalpy of the gelatinization peak of sorghum flour after heat treatment at 20% and 30% MC decreased significantly with increases in temperature of the heat treatment (Table 1.2). Low enthalpy of gelatinization of heat-treated flour was associated with low RS content of the samples after heat treatment at high MC and temperature. For samples with increased RS content, enthalpy was similar to that of the control sample. DSC results for similar heat-moisture treatment on starch (Table 1.3) also showed the same trend of increasing gelatinization temperature and decreasing enthalpy with increase in MC and intensity of heat treatment.

Amylose-lipid complex properties

Table 1.4 shows the thermal properties of the amylose-lipid complex of sorghum flour before and after different heat-moisture treatment conditions. Although no significant changes were detected in onset, peak, and conclusion temperatures of the amylose-lipid complex peak for samples from dry heat treatments (0% MC), the amylose-lipid complex peaks shifted to high temperatures for sample treated at 140^{o}C with 12.5 % MC, and samples treated at 120^{o}C and 140^{o}C with 20% and 30% MC. High moisture and temperature of heat treatments allowed the amylose to mobilize and re-associate to form a stronger amylose-lipid complex that melted at higher temperatures.

Crystallinity

Native sorghum flour has an A-type crystal pattern, displaying peaks at 2θ of 15, 17, 18, and 23^{o} (Fig. 1.7), which is typical for normal cereal starch (Zobel, 1988). Sorghum flour samples after heat treatment for 4 h at 120^{o}C with 20% MC, as well as 140^{o}C with 20% and 30% MC showed new peaks at 2θ angle of 7, 13, and 20, which are typical Vh-type X-ray diffraction pattern for amylose-lipid complex crystal (Zobel, 1988). For samples with high RS content (heat treatments for 4 h with 20% MC at 100^{o}C and 120^{o}C, and 12.5% MC at 140^{o}C), X-ray patterns were similar to the native flour. Thus, the increase in RS content of these samples cannot be explained by changes in crystallinity. A lower A-Type pattern of samples treated at 120^{o}C and 140^{o}C was in agreement with DSC results showing increases in the degree of gelatinization where starch crystal melted during heat treatments with high temperature and MC. After heat treatments with high temperature and moisture, amylose associated with lipid to form amylose lipid complex crystals, which showed the typical Vh-type X-ray pattern.

Sorghum starch after heat treatments at high MCs (20% and 30%) and high temperatures (120^{o}C and 140^{o}C) also had the Vh-type X-ray diffraction pattern (Figure 1.8). Changes in crystalinity of isolated sorghum starch and sorghum starch in flour after heat treatments were similar, but the changes in RS content of heat-treated sorghum flour were more (e.g from 5.6% to 22.1 %) than the change in RS content of heat-treated sorghum starch (9.6% to 15.2%). Thus, the increase in RS content of sorghum flour after heat treatment likely not correlated with changes in crystallinity.

Changes in sorghum protein after heat treatments

Protein digestibility

Protein digestibility of native flour and heat treated flour under different conditions is reported in Figure 1.9 as percentage of digested protein. Protein digestibility of sorghum flour decreased significantly after heat-moisture treatment (12.5%, 20%, and 30% MC) but not after the dry heat treatment (0% MC). Protein digestibility clearly decreased as treatment temperature increased for samples with 12.5% MC. With heat treatments at higher MC (20% and 30%), protein digestibility of the heat treated flour decreased substantially regardless of the temperature of the heat treatment. Cooking sorghum flour in the presence of water is well known to reduce protein digestibility due to increases in disulfide cross-links (Duodu, Nunes, Delgadillo, Parker, Mills, Belton, & Taylor, 2002). Decreased in sorghum protein digestibility has been observed after cooking of sorghum flour as reported by Hamaker et al. (1986), but the extent of decreased protein digestibility of cooked sorghum is different compared with heat-moisture treated sorghum flour. In a previous study, protein digestibility of uncooked sorghum flour changed from 80.7% to 64.8% after cooking for 20 min in boiling water with excess water (1:10). In contrast, protein digestibility of untreated sorghum flour changed from 67.8% to less than 10% after heat moisture treatments with 20% MC for 4 h. Duodu, Nunes, Delgadillo, Parker, Mills, Belton, & Taylor (2002) also reported decreases in protein digestibility (67.4% of uncooked white endosperm sorghum flour to 39.4% for cooked samples). Heat-moisture treatments appeared more effective in changing the protein, possibly because of the high temperature (>100^{o}C) and low MC compared with normal cooking (100^{o}C with excess water).

Protein extractability

As a results of different heat treatment conditions, heat treated sorghum protein was expected to form cross-linking polymeric proteins with larger size that could be extracted and observed on SEC chromatograms as peaks at a lower retention time compared with the native sorghum protein. SEC chromatograms (Figures 1.10 and 1.11) showed no significant change in retention time of non-reduced and reduced protein fractions before and after heat treatment. Quantitative changes in peak height and total area were observed. Sorghum protein after heat treatments became less extractable, and could not be seen on the SEC chromatograms. Similar to protein digestibility, protein extractability of sorghum flour after heat treatment generally

decreased with increases in temperature, time and MC of the heat treatment (Figure 1.12). For all samples with high RS contents (treatments at 100^{o}C and 120^{o}C with 20% MC for 4 h, and treatment at 140^{o}C with 12.5% MC for 4 h), protein extractability was substantially lower (21.5%, 8.4% and 19.9%) than for the native flour (assuming 100% protein was extracted from the native flour). Heat-moisture treatments appeared to have a major effect on sorghum protein solubility. It is widely accepted that sorghum protein subjected to heat treatment will form disulfide cross-links that make the protein less digestible. Because water is limited during heat-moisture treatments (less than 30%), sorghum protein seems to have the optimum amount of water and minimal mobility to facilitate cross-linking, and make sorghum protein extremely inaccessible for enzyme or reducing agents to act on.

Protein surface hydrophobicity

Protein surface hydrophobicity of protein in native flour and flour after heat treatments is shown in Figure 1.13. The dry heat treatments did not change hydrophybicity of the protein in sorghum flour. After heat-moisture treatments with 12.5% MC, protein surface hydrophobicity decreased with increases in time and temperature of the treatments. Longer heat-moisture treatments (4 h) at high temperatures allowed the protein in the flour to re-associate, with the more hydrophilic sites on the surface and more hydrophobic sites folding inside. Thus, the protein surface hydrophobicity decreased after heat-moisture treatments. When MC increased to 20% and 30%, protein hydophobicity did not decrease as much as protein digestibility or protein extractability of the same treatments.

FTIR

The secondary structures of protein can be estimated by FTIR, with a characteristic band of α-helix structure around 1660 cm^{-1} and a characteristic band of β-sheet structure around 1620 cm^{-1} (Surewicz & Mantsch, 1988). Sorghum flour after dry heat treatment had only the characteristic band 1660 cm^{-1} representing the α-helix structure of protein (Figure 1.14). After heat-moisture treatments with MC of 12.5%, 20%, and 30%, the characteristic band at 1620 cm^{-1} appeared on all the samples. The FTIR results were in agreement with the protein digestibility and solubility data, and confirmed that dry heat treatments did not effectively alter the protein properties and structures, but that the heat-moisture treatments changed sorghum protein. The β-sheet structure

developed during heat-treatments could be a factor that made sorghum protein less digestible and more difficult to extract.

Conclusions

Heat-moisture treatment can effectively increase RS content of sorghum flour by modifying sorghum protein, presumably through increases in cross-linking. Changes in sorghum starch after heat-moisture treatment were minimal, but significant decreases in both protein digestibility and protein extractability were probably due to disulfide bond formation in sorghum protein during treatment. Unlike with sorghum flour, the same heat-moisture treatment of isolated starch did not result in increased RS content at the same level. This study provides insight into how sorghum protein and starch changes during heat-moisture treatment, providing information for further modification to increase or decrease digestibility of sorghum protein and starch without affecting starch structures and functionality. Further investigation of the changes in starch and protein at molecular level by nuclear magnetic resonance (NMR) spectroscopy would help to understand if new chemical bonds are forming in the protein or between starch and protein after heat-moisture treatments. Confocal microscopy on the isolated starch would show how the protein distribution before and after heat treatment.

References

Adebowalea, K., Olu-Owolabi, B., Olayinka, O., & Lawal, O. (2005). Effect of heat-moisture treatment and annealing on physicochemical properties of red sorghum starch. *African Journal of Biotechnology*, *4*(9)

Amadou, I., Gounga, M. E., Shi, Y., & Le, G. (2014). Fermentation and heat-moisture treatment induced changes on the physicochemical properties of foxtail millet (< i> setaria italica</i>) flour. *Food and Bioproducts Processing*, *92*(1), 38-45.

Bauer-Marinovic, M., Florian, S., Müller-Schmehl, K., Glatt, H., & Jacobasch, G. (2006). Dietary resistant starch type 3 prevents tumor induction by 1, 2-dimethylhydrazine and alters proliferation, apoptosis and dedifferentiation in rat colon. *Carcinogenesis*, *27*(9), 1849-1859.

Behall, K., Scholfield, D., Hallfrisch, J., & Liljeberg-Elmstahl, H. (2006). Consumption of both resistant starch and beta-glucan improves postprandial plasma glucose and insulin in women. *Diabetes Care*, *29*(5), 976-981.

Brumovsky, J. O., & Thompson, D. B. (2001). Production of boiling-stable granular resistant starch by partial acid hydrolysis and hydrothermal treatments of high-amylose maize starch. *Cereal Chemistry, 78*(6), 680-689.

Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. *Meat Science, 74*(4), 681-683.

Chung, H., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers, 75*(3), 436-447.

Duodu, K., Nunes, A., Delgadillo, I., Parker, M., Mills, E., Belton, P., & Taylor, J. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science, 35*(2), 161-174.

Duodu, K., Nunes, A., Delgadillo, I., Parker, M., Mills, E., Belton, P., & Taylor, J. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science, 35*(2), 161-174.

Elkin, R. G., Arthur, E., Hamaker, B. R., Axtell, J. D., Douglas, M. W., & Parsons, C. M. (2002). Nutritional value of a highly digestible sorghum cultivar for meat-type chickens circle times. *Journal of Agricultural and Food Chemistry, 50*(14), 4146-4150.

Englyst, H., Kingman, S., & Cummings, J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, S33-S50.

Gibson, T., Solah, V., & McCleary, B. (1997). A procedure to measure amylose in cereal starches and flours with concanavalin A. *Journal of Cereal Science, 25*(2), 111-119

Hamaker, B. R., Kirleis, A. W., Mertz, E. T., & Axtell, J. D. (1986). Effect of cooking on the protein profiles and invitro digestibility of sorghum and maize. *Journal of Agricultural and Food Chemistry, 34*(4), 647-649.

Hasjim, J., Lee, S., Hendrich, S., Setiawan, S., Ai, Y., & Jane, J. (2010). Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. *Cereal Chemistry, 87*(4), 257-262.

Hoover, R., & Vasanthan, T. (1994). Effect of heat-moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches. *Carbohydrate Research, 252*, 33-53.

Ioerger, B., Bean, S. R., Tuinstra, A. R., Pedersen, J. F., Erpelding, J., Lee, K. A., & Herrman, T. J. (2007). Characterization of polymeric proteins from vitreous and floury sorghum endosperm. *Journal of Agricultural and Food Chemistry, 55*(25), 10232-10239.

Jenkins, D. J., Vuksan, V., Kendall, C. W., Würsch, P., Jeffcoat, R., Waring, S., . . . Wong, E. (1998). Physiological effects of resistant starches on fecal bulk, short chain fatty acids, blood lipids and glycemic index. *Journal of the American College of Nutrition, 17*(6), 609-616.

Jiranuntakul, W., Puttanlek, C., Rungsardthong, V., Puncha-Arnon, S., & Uttapap, D. (2011). Microstructural and physicochemical properties of heat-moisture treated waxy and normal starches. *Journal of Food Engineering, 104*(2), 246-258.

Keenan, M. J., Zhou, J., McCutcheon, K. L., Raggio, A. M., Bateman, H. G., Todd, E., . . . Martin, R. J. (2006). Effects of resistant starch, A Non-digestible fermentable fiber, on reducing body fat. *Obesity, 14*(9), 1523-1534.

Kim, J., & Huber, K. C. (2013). Heat–moisture treatment under mildly acidic conditions alters potato starch physicochemical properties and digestibility. *Carbohydrate Polymers, 98*(2), 1245-1255.

Lin, J., Singh, H., Wen, C., & Chang, Y. (2011). Partial-degradation and heat-moisture dual modification on the enzymatic resistance and boiling-stable resistant starch content of corn starches. *Journal of Cereal Science, 54*(1), 83-89.

Maki, K. C., Pelkman, C. L., Finocchiaro, E. T., Kelley, K. M., Lawless, A. L., Schild, A. L., & Rains, T. M. (2012). Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. *The Journal of Nutrition, 142*(4), 717-723.

Mertz, E. T., Hassen, M. M., Cairnswhittern, C., Kirleis, A. W., Tu, L., & Axtell, J. D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences, 81*(1), 1-2.

Gibson, T., Solah, V., & McCleary, B. (1997). A procedure to measure amylose in cereal starches and flours with concanavalin A. *Journal of Cereal Science, 25*(2), 111-119

Niba, L. L. (2003). Processing effects on susceptibility of starch to digestion in some dietary starch sources. *International Journal of Food Sciences and Nutrition, 54*(1), 97-109.

Olayinka, O. O., Adebowale, K. O., & Olu-Owolabi, B. I. (2008). Effect of heat-moisture treatment on physicochemical properties of white sorghum starch. *Food Hydrocolloids, 22*(2), 225-230.

Reddy, C. K., Suriya, M., & Haripriya, S. (2013). Physico-chemical and functional properties of resistant starch prepared from red kidney beans (< i> phaseolus vulgaris. L</i>) starch by enzymatic method. *Carbohydrate Polymers,*

Rooney, L. W., & Pflugfelder, R. L. (1986). Factors affecting starch digestibility with special emphasis on sorghum and corn. *Journal of Animal Science, 63*(5), 1607-1623.

Sajilata, M., Singhal, R., & Kulkarni, P. (2006). Resistant starch - A review. *Comprehensive Reviews in Food Science and Food Safety, 5*(1), 1-17.

Sang, Y., & Seib, P. A. (2006). Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation. *Carbohydrate Polymers, 63*(2), 167-175.

Sankhon, A., Yao, W., Amadou, I., Wang, H., Qian, H., & Sangare, M. (2013). Effect of pyrodextrinization, crosslinking and heat-moisture treatment on in vitro formation and digestibility of resistant starch from african locust bean (parkia biglobosa). *Tropical Journal of Pharmaceutical Research, 12*(2), 173-179.

Shi, Y., & Trzasko, P. T. (1997). *Process for Producing Amylase Resistant Granular Starch.* Patent # US5593503 A

Shin, S. I., Kim, H. J., Ha, H. J., Lee, S. H., & Moon, T. W. (2005). Effect of hydrothermal treatment on formation and structural characteristics of slowly digestible non-pasted granular sweet potato starch. *Starch-Starke, 57*(9), 421-430.

Singh, H., Chang, Y. H., Lin, J., Singh, N., & Singh, N. (2011). Influence of heat–moisture treatment and annealing on functional properties of sorghum starch. *Food Research International, 44*(9), 2949-2954.

Sun, Q., Han, Z., Wang, L., & Xiong, L. (2014). Physicochemical differences between sorghum starch and sorghum flour modified by heat-moisture treatment. *Food Chemistry, 145*, 756-764.

Surewicz, W. K., & Mantsch, H. H. (1988). New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochimica Et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 952*, 115-130.

Varatharajan, V., Hoover, R., Liu, Q., & Seetharaman, K. (2010). The impact of heat-moisture treatment on the molecular structure and physicochemical properties of normal and waxy potato starches. *Carbohydrate Polymers, 81*(2), 466-475.

Wepner, B., Berghofer, E., Miesenberger, E., Tiefenbacher, K., & NK Ng, P. (1999). Citrate starch—Application as resistant starch in different food systems. *Starch-Stärke, 51*(10), 354-361.

Wongsagonsup, R., Varavinit, S., & BeMiller, J. N. (2008). Increasing slowly digestible starch content of normal and waxy maize starches and properties of starch products. *Cereal Chemistry, 85*(6), 738-745.

Woo, K., & Seib, P. (2002). Cross-linked resistant starch: Preparation and properties 1. *Cereal Chemistry, 79*(6), 819-825.

Xie, X. S., & Liu, Q. (2004). Development and physicochemical characterization of new resistant citrate starch from different corn starches. *Starch-Stärke, 56*(8), 364-370.

Zavareze, E. d. R., & Dias, A. R. G. (2011). Impact of heat-moisture treatment and annealing in starches: A review. *Carbohydrate Polymers, 83*(2), 317-328.

Zhang, G. Y., & Hamaker, B. R. (2003). A three component interaction among starch, protein, and free fatty acids revealed by pasting profiles. *Journal of Agricultural and Food Chemistry, 51*(9), 2797-2800.

Zobel, H. (1988). Starch crystal transformations and their industrial importance. *Starch-Stärke, 40*(1), 1-7.

Tables and Figures

Table 1.1. Resistant starch content of sorghum flour and isolated sorghum starch with and without heat treatment for 4hr with 20% MC at 100°C

Samples	Resistant starch contents
Sorghum flour	5.6±2.6
Heat treated sorghum flour (4hr, 20% MC, 100°C)	22.1±1.1
Isolated sorghum starch	9.6±0.3
Heat treated isolated sorghum starch (4hr, 20% MC, 100°C)	15.2±2.2
Isolated starch from heat treated sorghum flour (4hr, 20% MC, 100°C)	30.2±4.8

Table 1.2. Thermal properties of sorghum flour after 4 h of heat treatments with different MC (0%, 12.5%, 20%, and 30%) and temperatures (100°C, 120°C, and140°C) as determined by differential scanning calorimetry[A]

Treatment conditions		T_0* (°C)	T_p* (°C)	T_c* (°C)	ΔH** (J/g)
Native (no heat treatment)		65.4±0.5^{g}	73.4±0.5^{f,g,h}	87.6±1.5^{f,g}	7.1±0.4^{b,c}
0% MC	100°C	64.9±0.0^{g,h}	74.4±0.2^{e}	88.6±0.1^{f,g}	6.6±0.2^{c,d,e}
	120°C	64.0±0.1^{h,i}	73.2±0.3$^{g.h}$	88.9±0.1^{f,g}	6.0±0.0^{e,f}
	140°C	63.5±0.1^{i}	72.7±0.2^{h}	89.1±0.6^{f}	5.9±0.2^{f}
12.5% MC	100°C	65.2±0.0^{g}	74.2±0.0^{e,f,g}	87.8±0.2^{f,g}	6.7±0.1^{b,c,d}
	120°C	65.3±0.6^{g}	74.4±0.3^{e,f}	87.2±0.4^{g}	6.0±0.4^{e,f}
	140°C	69.3±0.7^{f}	78.4±0.2^{d}	91.6±0.1^{e}	7.2±0.3^{b}
20% MC	100°C	70.1±1.1^{f}	77.7±0.6^{d}	88.9±0.3^{f,g}	7.3±0.4^{b}
	120°C	72.4±0.4^{e}	81.8±0.8^{c}	96.7±0.5^{d}	6.4±0.2^{d,e,f}
	140°C	77.2±0.1^{c}	86.6±0.0^{b}	101.5±0.6^{b}	3.5±0.2^{g}
30% MC	100°C	74.1±1.3^{d}	86.5±0.1^{b}	98.4±0.3^{c}	5.8±0.3^{f}
	120°C	79.1±0.3^{b}	87.0±0.1^{b}	100.2±0.3^{b}	1.7±0.0^{h}
	140°C	83.2±0.2^{a}	90.8±1.3^{a}	105.6±0.7^{a}	0.2±0.1^{i}

* T_0, Tp, and Tc indicate onset, peak, and conclusion temperature of gelatinization. ** ΔH indicates enthalpy of gelatinization

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 1.3. Thermal properties of sorghum starch after 4 h of heat treatments with different MC (0%, 12.5%, 20%, and 30%) and temperatures (100°C, 120°C, and140°C) as determined by differential scanning calorimetry[A]

Treatment conditions		T_0 * (°C)	T_p* (°C)	T_c* (°C)	ΔH ** (J/g)
Native (no heat treatment)		64.1±0.4^{d}	69.8±0.3^{e}	84.6±1.1^{d,e}	12.9±0.3^{a}
0% MC	100°C	63.2±0.8^{d,e}	69.9±1.3^{e}	82.3±0.2^{f}	11.1±1.4^{b,c}
	120°C	61.8±0.3^{e}	68.5±0.1^{f}	83.4±0.1^{e,f}	10.8±0.5^{b,c}
	140°C	60.0±0.5^{f}	67.3±0.4^{g}	83.8±0.1^{e,f}	10.4±0.4^{c,d}
9.8% MC	100°C	63.3±0.2^{d,e}	70.0±0.1^{e}	83.8±0.1^{e,f}	12.0±0.4^{a,b}
	120°C	63.9±0.3^{d}	70.5±0.0^{e}	84.9±0.2^{d,e}	11.6±0.2^{a,b,c}
	140°C	63.9±0.8^{d}	70.8±0.6^{e}	85.8±1.3^{c,d}	11.7±0.2^{a,b,c}
20% MC	100°C	66.1±0.1^{c}	75.1±0.1^{d}	86.5±0.9^{c}	11.5±1.2^{b,c}
	120°C	67.0±0.2^{c}	77.3±1.0^{c}	92.2±0.4^{b}	9.3±0.9^{d}
	140°C	68.9±0.0^{b}	77.6±0.0^{c}	94.6±0.3^{a}	7.0±0.2^{e}
30% MC	100°C	70.8±1.8^{a}	84.9±0.3^{a}	92.6±0.0^{b}	6.5±0.1^{e}
	120°C	72.3±2.0^{a}	78.8±1.1^{b}	95.6±0.2^{a}	1.7±0.3^{f}

* T_0, Tp, and Tc indicate onset, peak, and conclusion temperature of gelatinization. ** ΔH indicates enthalpy of gelatinization

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 1.4. Thermal properties of the amylose-lipid complex peak of sorghum flours after heat treatments for 4 h at different times and temperatures [A]

Treatment conditions		T_0 * (^oC)	T_p* (^oC)	T_c* (^oC)	ΔH ** (J/g)
Native (no heat treatment)		94.7±0.5^{h}	104.0±0.0^{d}	112.5±0.4^{g,h}	0.90±0.02^{d}
0 % MC	100^{o}C	95.8±0.3^{g,h}	103.9±0.3^{d,e}	113.3±0.0^{g}	0.60±0.03^{e}
	120^{o}C	97.1±0.0^{e,f,g}	103.8±0.6^{d,e}	111.4±0.1^{h,i}	0.42±0.02^{e}
	140^{o}C	96.2±0.9^{f,g,h}	104.4±0.3^{d}	115.0±0.1^{f}	0.41±0.05^{e}
12.5 % MC	100^{o}C	96.2±0.1^{f,g,h}	103.4±0.1^{d,e}	110.6±0.9^{i}	0.35±0.02^{e}
	120^{o}C	95.3±0.6^{h}	102.8±0.2^{e}	111.1±0.1^{i}	0.41±0.02^{e}
	140^{o}C	111.1±0.3^{d}	119.9±0.3^{c}	133.4±0.2^{c}	1.56±0.02^{c}
20% MC	100^{o}C	97.6±0.5^{e,f}	103.5±0.3^{d,e}	111.2±0.2^{i}	0.34±0.1^{e}
	120^{o}C	113.7±2.1^{c}	119.4±1.5^{c}	128.7±1.5^{e}	0.45±0.12^{e}
	140^{o}C	115.3±0.5^{b}	125.7±0.1^{b}	137.4±0.2^{b}	2.42±0.38^{a}
30% MC	100^{o}C	98.2±0.4^{e}	104.2±0.3^{d}	111.7±0.0^{h,i}	0.37±0.01^{e}
	120^{o}C	113.0±0.1^{c}	120.2±0.1^{c}	131.6±0.0^{d}	1.86±0.09^{b}
	140^{o}C	117.8±0.0^{a}	127.5±0.0^{a}	138.6±0.3^{a}	2.66±0.02^{a}

* T_0, Tp, and Tc indicate onset, peak, and conclusion temperature of the melting of amylase-lipid complex ** ΔH indicates enthalpy of the melting of amylase-lipid complex

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 1.5. Crystalinity of sorghum flour after heat treatments for 4 h at different MCs and temperatures

Treatment conditions		A-type crystalinity (%)	Vh-type crystalinity (%)	Total crystalinity (%)
Native (no heat treatment)		21.7	3.0	24.7
0% MC	100°C	23.1	3.2	26.3
	120°C	20.9	2.5	23.3
	140°C	23.1	3.2	26.3
12.5% MC	100°C	21.9	3.0	24.9
	120°C	22.8	3.5	26.3
	140°C	20.1	4.1	24.2
20% MC	100°C	22.0	3.9	25.9
	120°C	19.5	4.4	23.8
	140°C	17.5	8.5	26.0
30% MC	100°C	19.9	5.1	25.0
	120°C	16.7	8.0	24.7
	140°C	12.8	8.3	21.1

Table 1.6. Crystalinity of sorghum starch after heat treatments for 4 h at different MCs and temperatures

Treatment conditions		A-type crystalinity (%)	Vh-type crystalinity (%)	Total crystalinity (%)
Native (no heat treatment)		24.5	3.2	27.7
0% MC	100°C	29.6	4.2	33.8
	120°C	26.5	3.7	30.2
	140°C	26.7	3.5	26.7
9.8% MC	100°C	28.5	3.8	32.3
	120°C	31.7	5.4	37.1
	140°C	25.6	3.7	29.3
20% MC	100°C	19.2	2.3	21.6
	120°C	24.0	5.3	29.3
	140°C	19.6	7.5	27.2
30% MC	100°C	26.0	7.2	33.2
	120°C	16.2	7.0	23.3

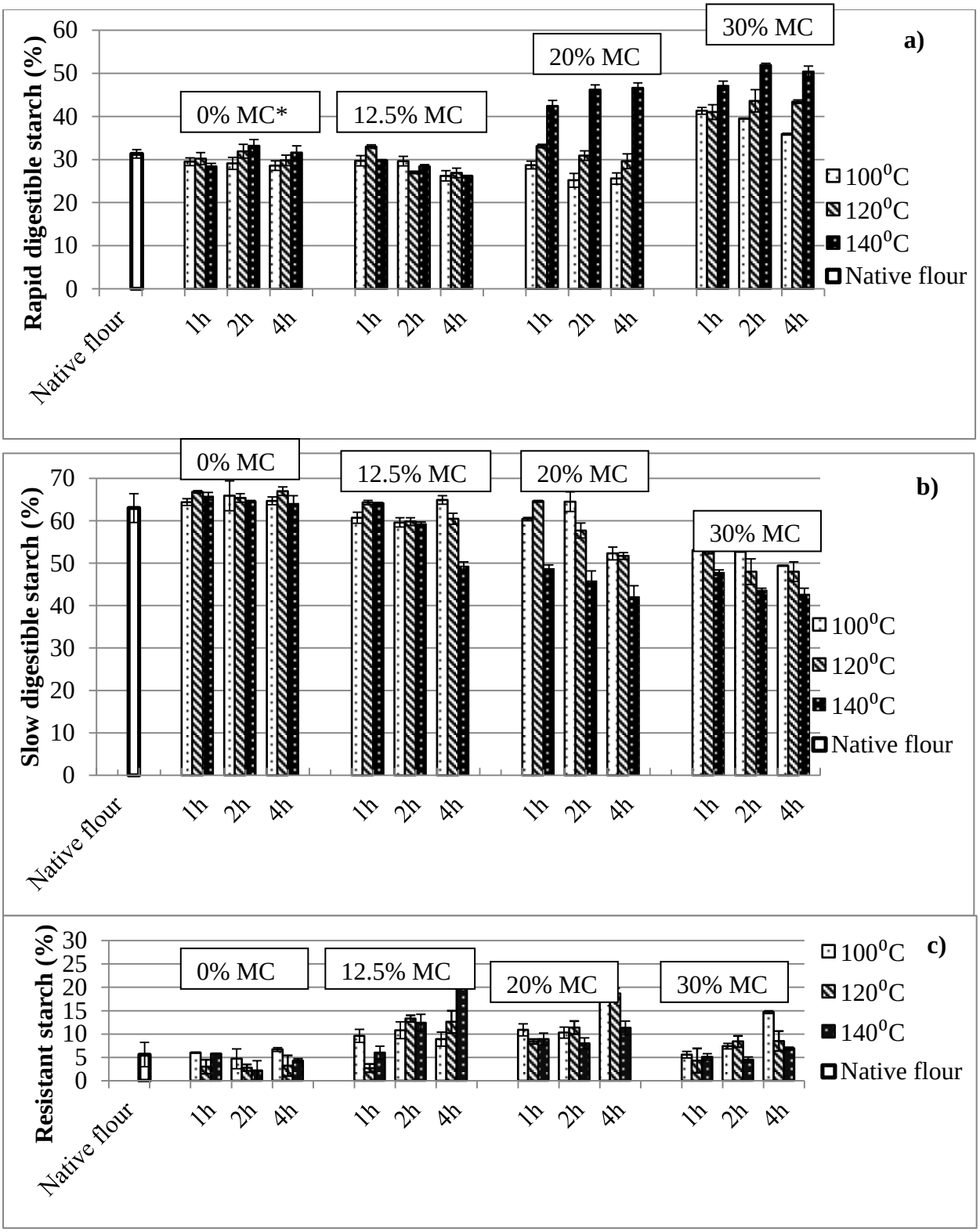


Figure 1.1 Rapid digestible starch (a), slow digestible starch (b), and resistant starch (c) content of sorghum flour after heat treatments at different temperatures, moisture contents (*) and time intervals.

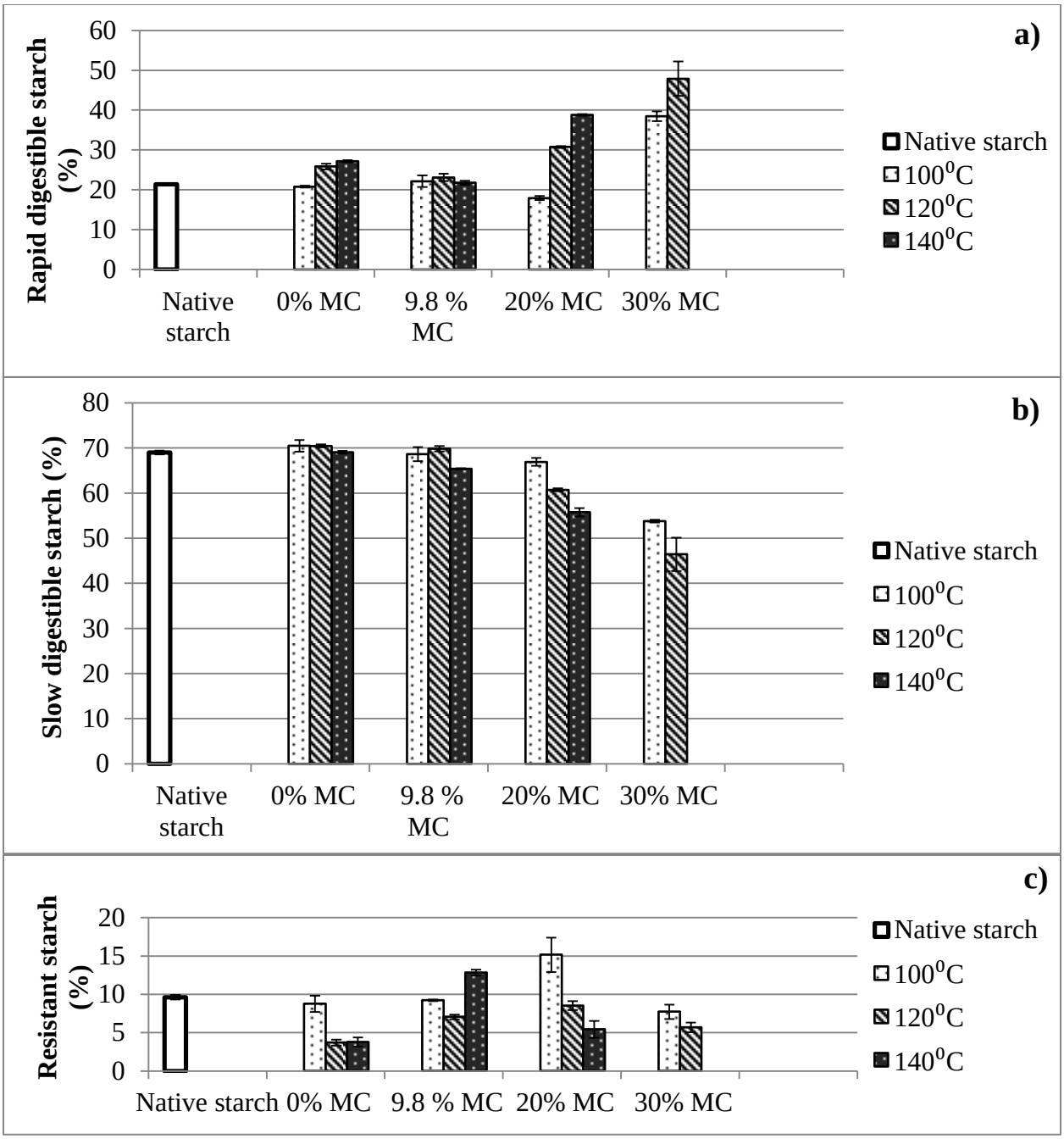


Figure 1.2 Rapid digestible starch (a), slowly digestible starch (b), and resistant starch (c) of sorghum starch after 4-hour heat treatments at different time (100°C, 120°C, and 140°C) with different MCs (0%, 9.8%, 20%, and 30%)

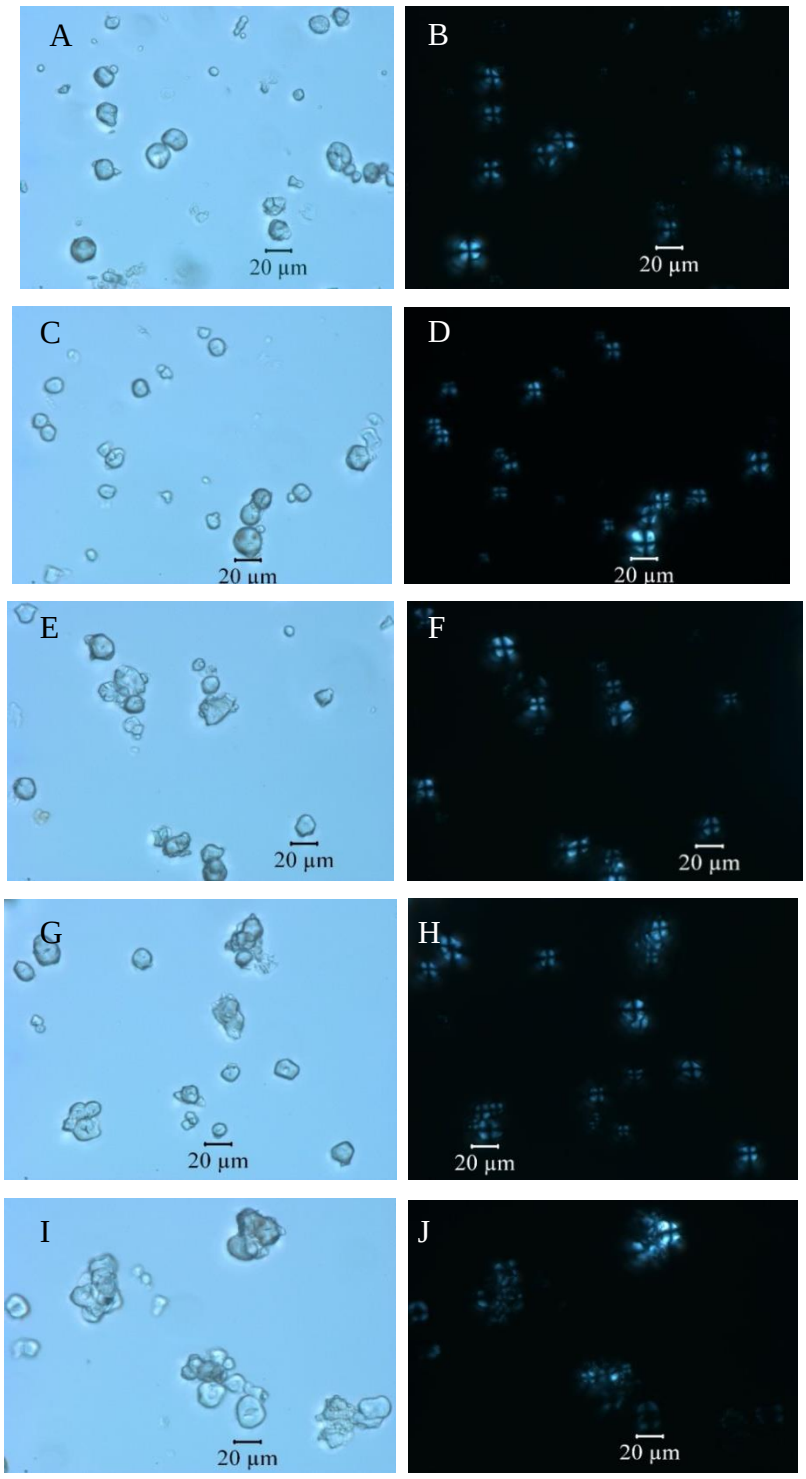


Figure 1.3. Microscopic images of native sorghum flour (A, B) and sorghum flour after heat treatments for 4 h at 100°C with MC of 0% (C, D), 12.5% (E, F), 20% (G, H), and 30% (I, J) viewed under brightfield transmitted light (A, C, E, G, I) and poralized light (B, D, F, H, J)

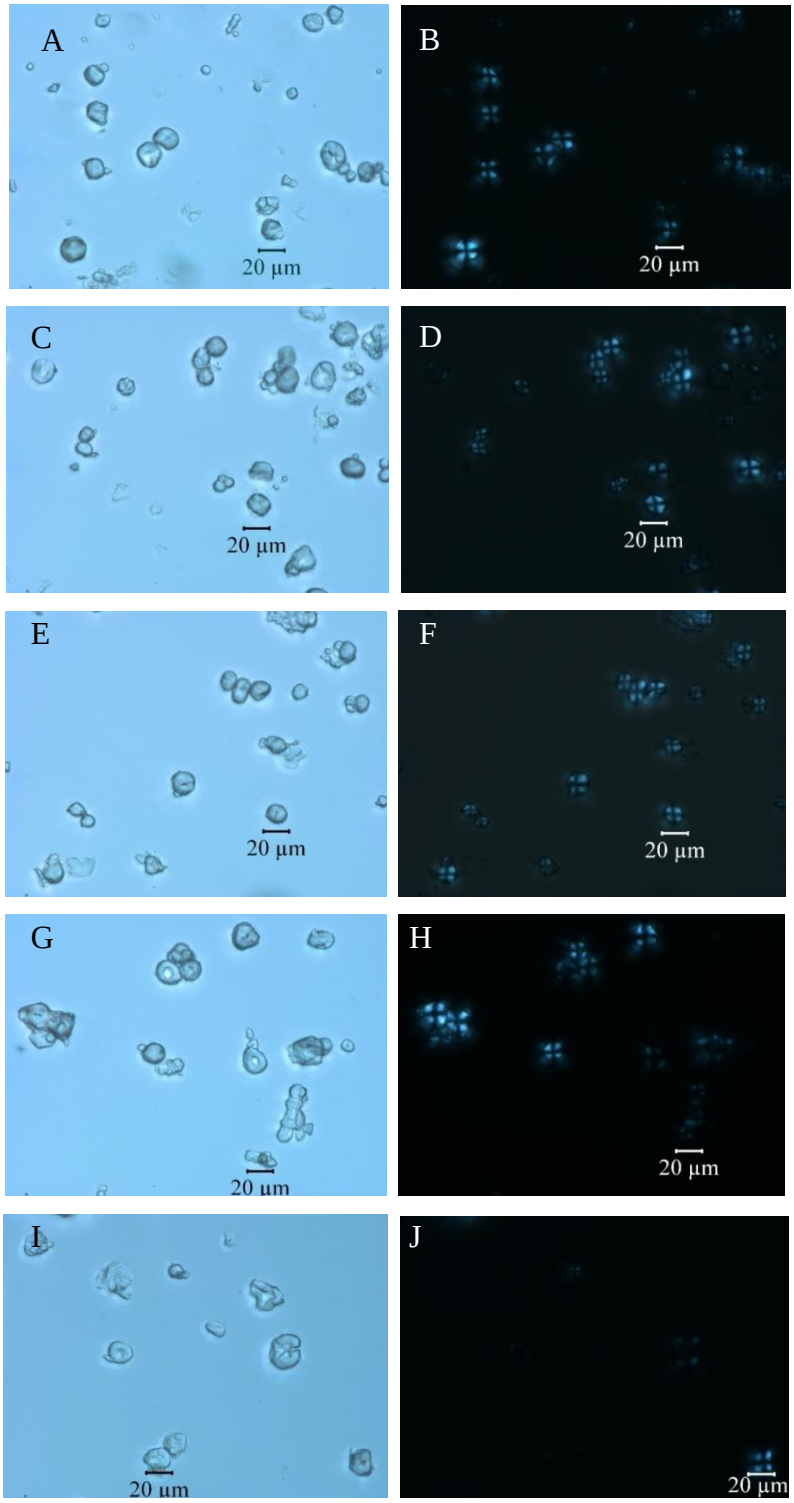


Figure 1.4. Microscopic images of native sorghum flour (A, B) and sorghum flour after heat treatments for 4 h at 120°C with MC of 0% (C, D), 12.5% (E, F), 20% (G, H), and 30% (I, J) viewed under brightfield transmitted light (A, C, E, G, I) and poralized light (B, D, F, H, J)

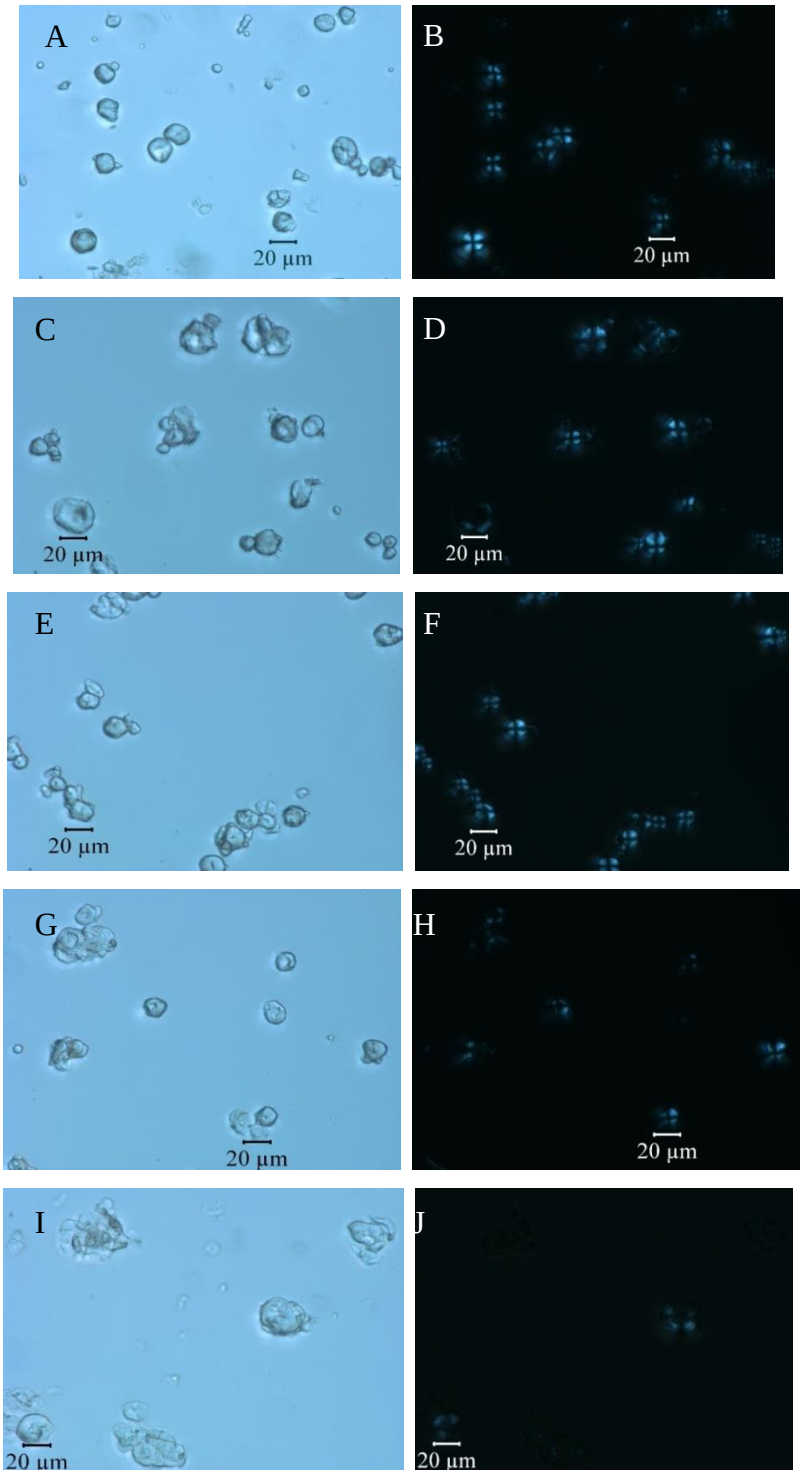


Figure 1.5. Microscopic images of native sorghum flour (A, B) and sorghum flour after heat treatments for 4 h at 140°C with MC of 0% (C, D), 12.5% (E, F), 20% (G, H), and 30% (I, J) viewed under brightfield transmitted light (A, C, E, G, I) and poralized light (B, D, F, H, J)

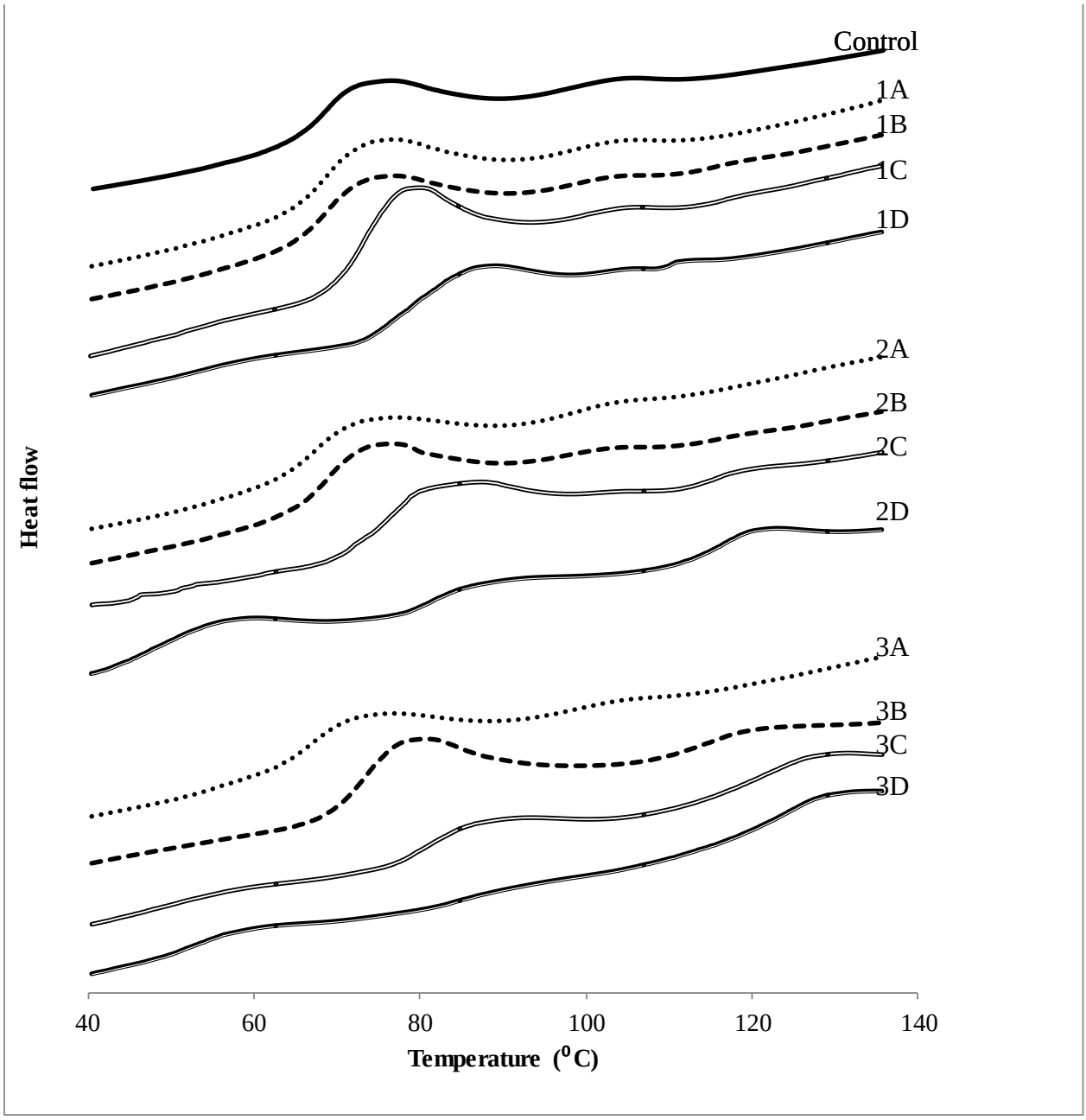


Figure 1.6. DSC thermogram of sorghum flour samples after 4 h of heat treatments at different temperatures 100°C (1), 120°C (2), and 140°C (3) and MCs 0% (A), 12.5% (B), 20%(C), and 30% (D)

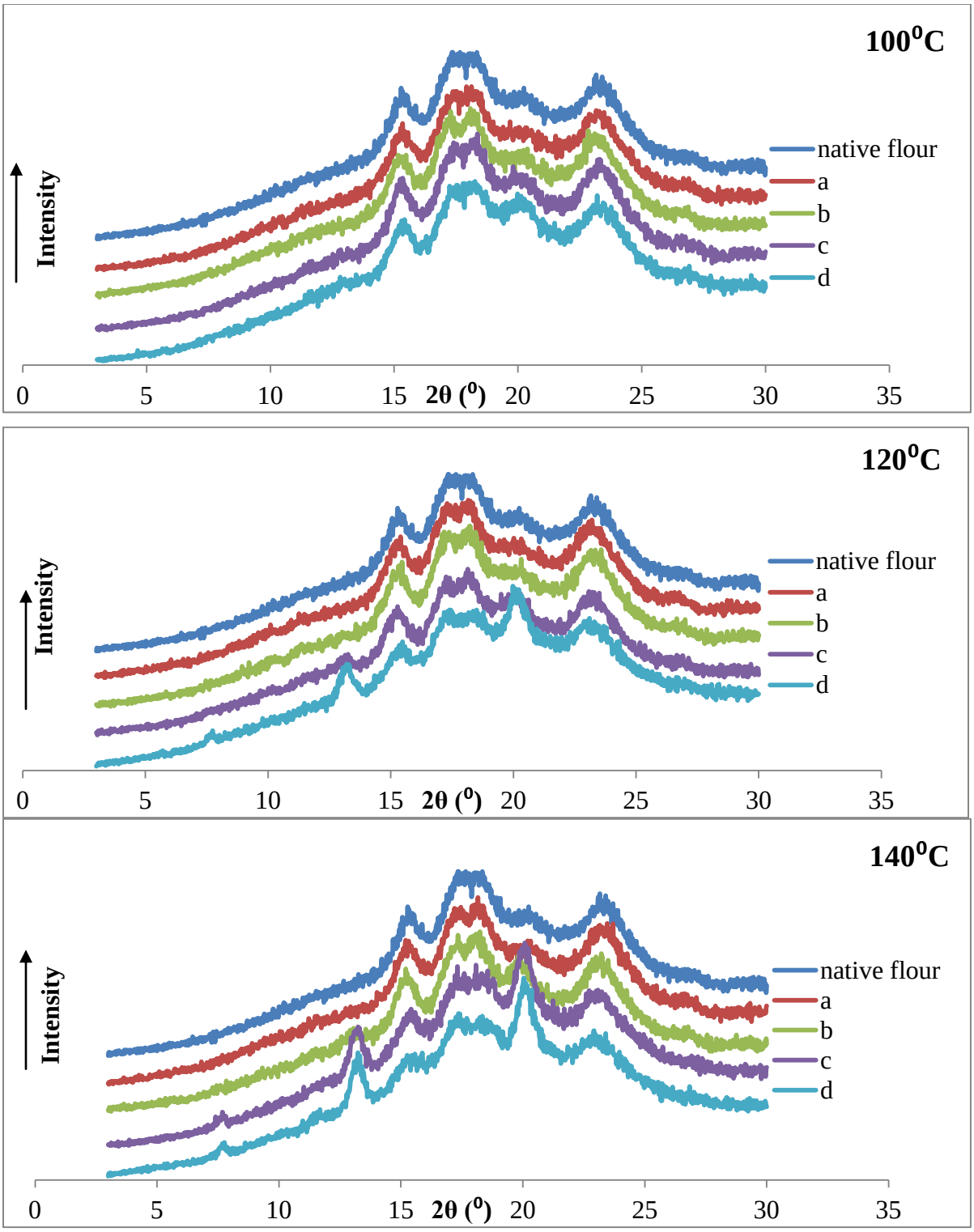


Figure 1.7. X-ray diffractogram of sorghum flour after 4-hour heat treatments at different temperatures (100°°C, 120°C, and 140°C) and MCs (a-0%, b-12.5%, c-20%, d-30%)

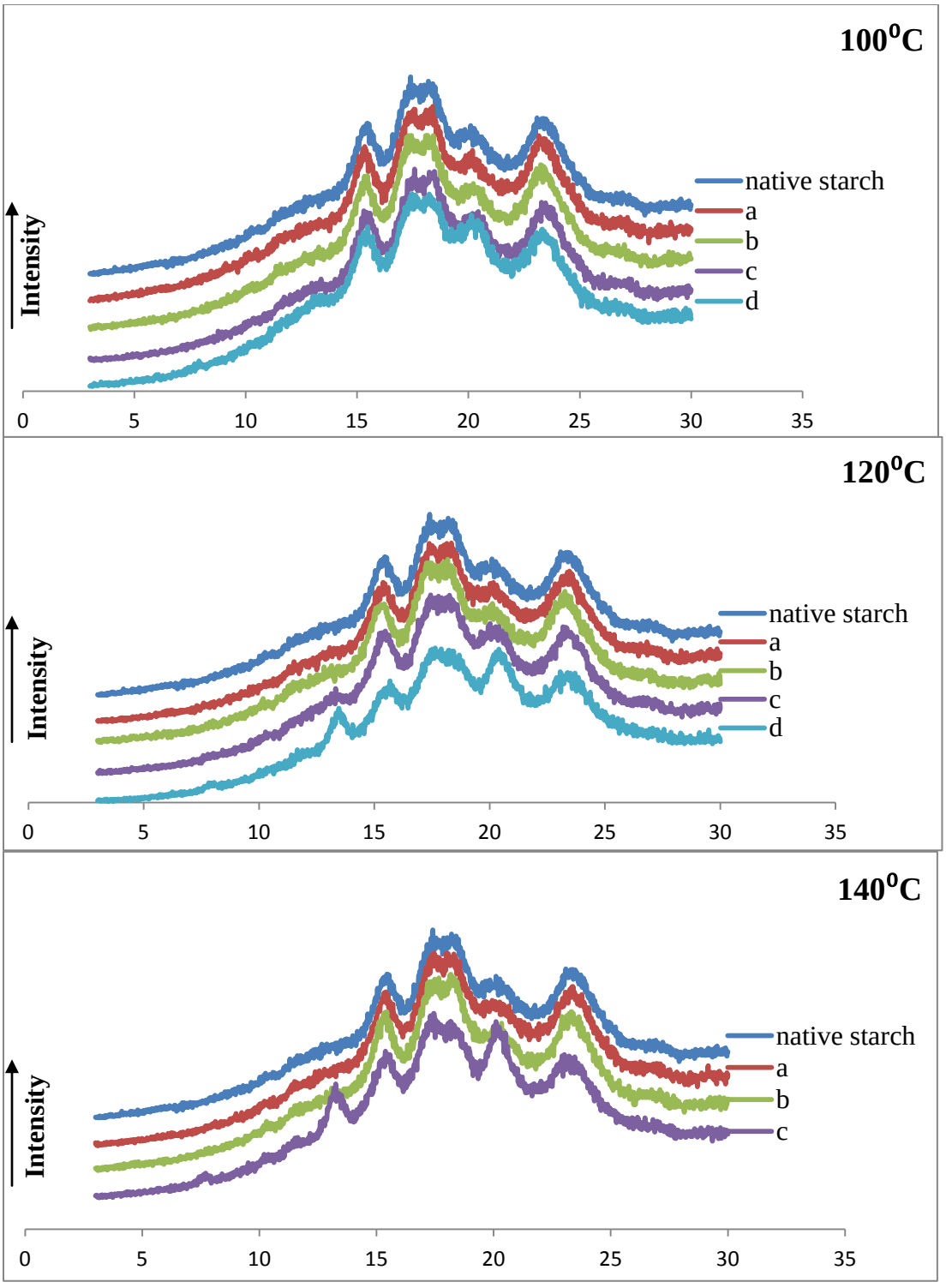


Figure 1.8. X-ray diffractogram of sorghum starch after 4-hour heat treatments at different temperatures and MCs (100°C, 120°C, and 140°C) and MCs (a-0%, b-12.5%, c-20%, d-30%)

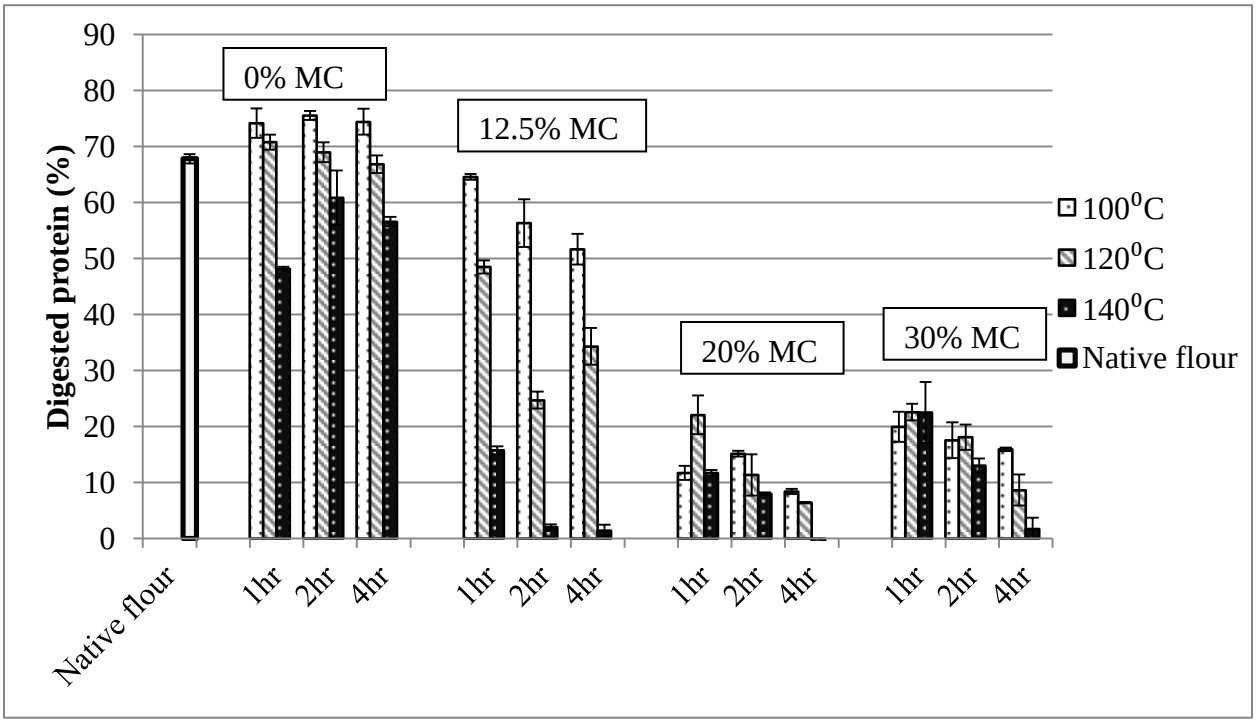
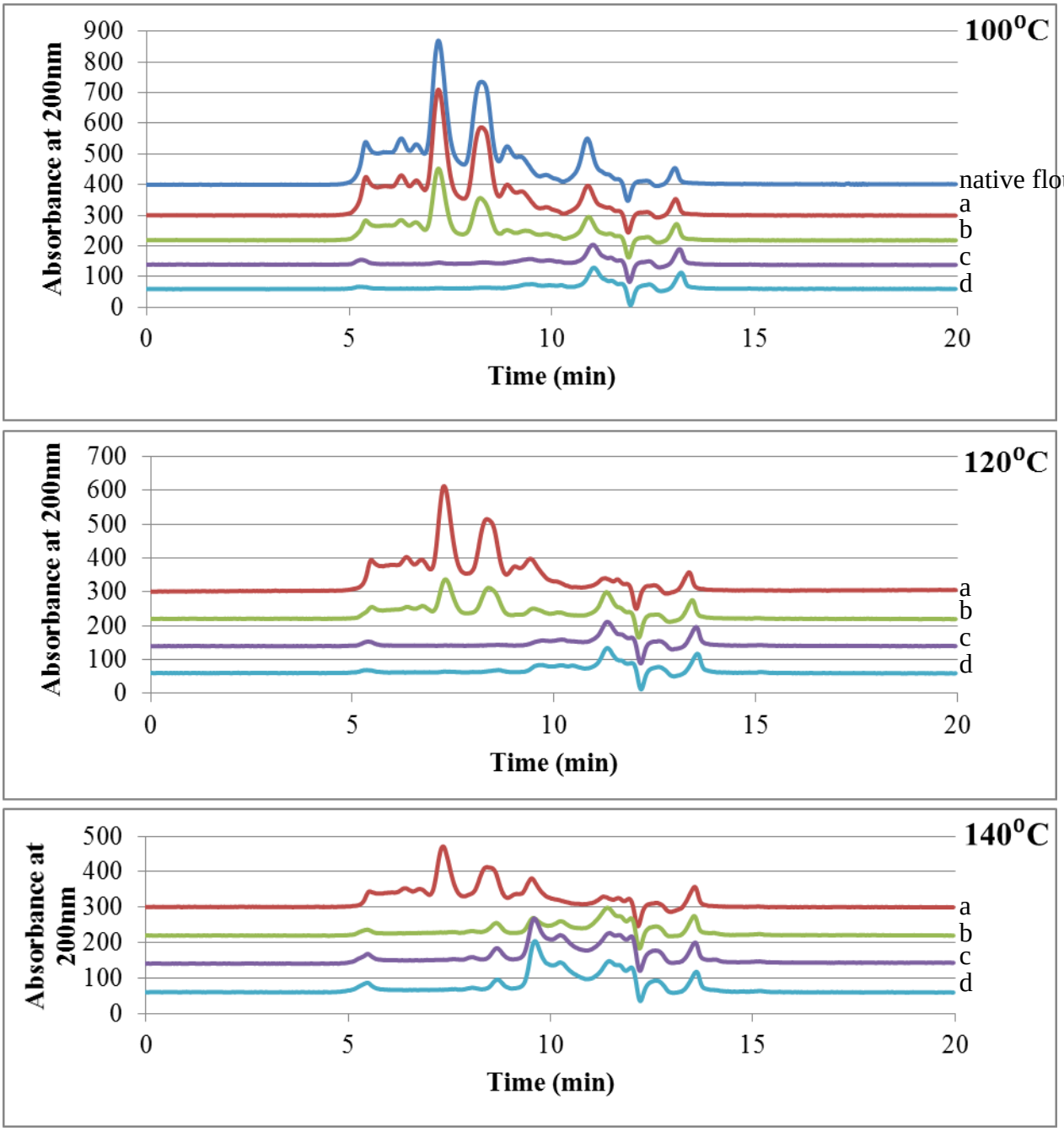
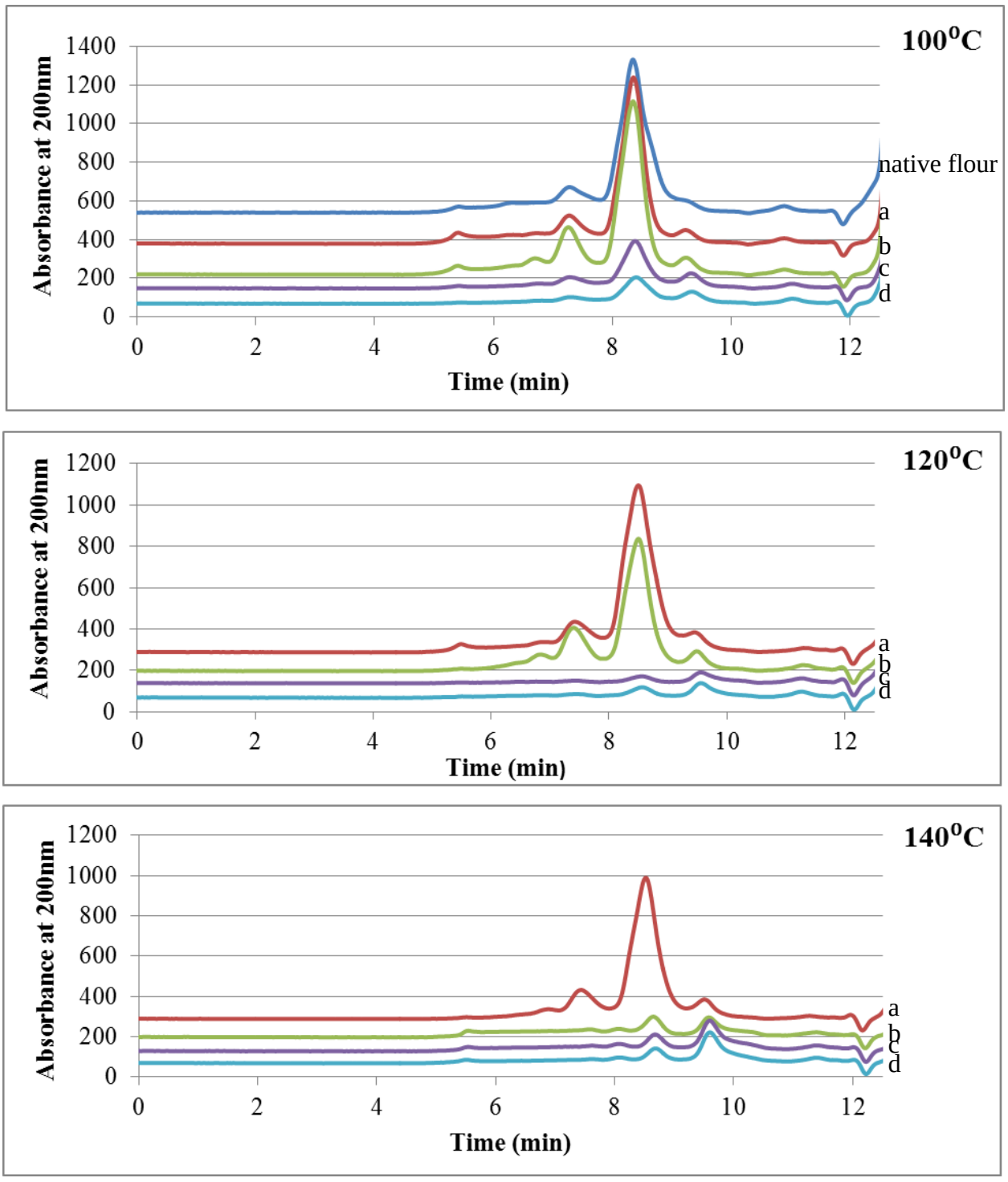


Figure 1.9. Protein digestibility of sorghum flour after heat treatments at different times, temperatures and MCs



1.10. HPLC chromatographs of non-reduced protein fraction extracted from native flour and heat treated flour at 100°C, 120°C, and 140°C for 4 hr at different MC (a- 0%, b- 12.5%, c- 20%, d-30%)



1.11. HPLC chromatographs of reduced protein fraction extracted from native flour and heat treated flour at 100°C, 120°C, and 140°C for 4 hr at different MC (a- 0%, b-12.5%, c-20%, d-30%)

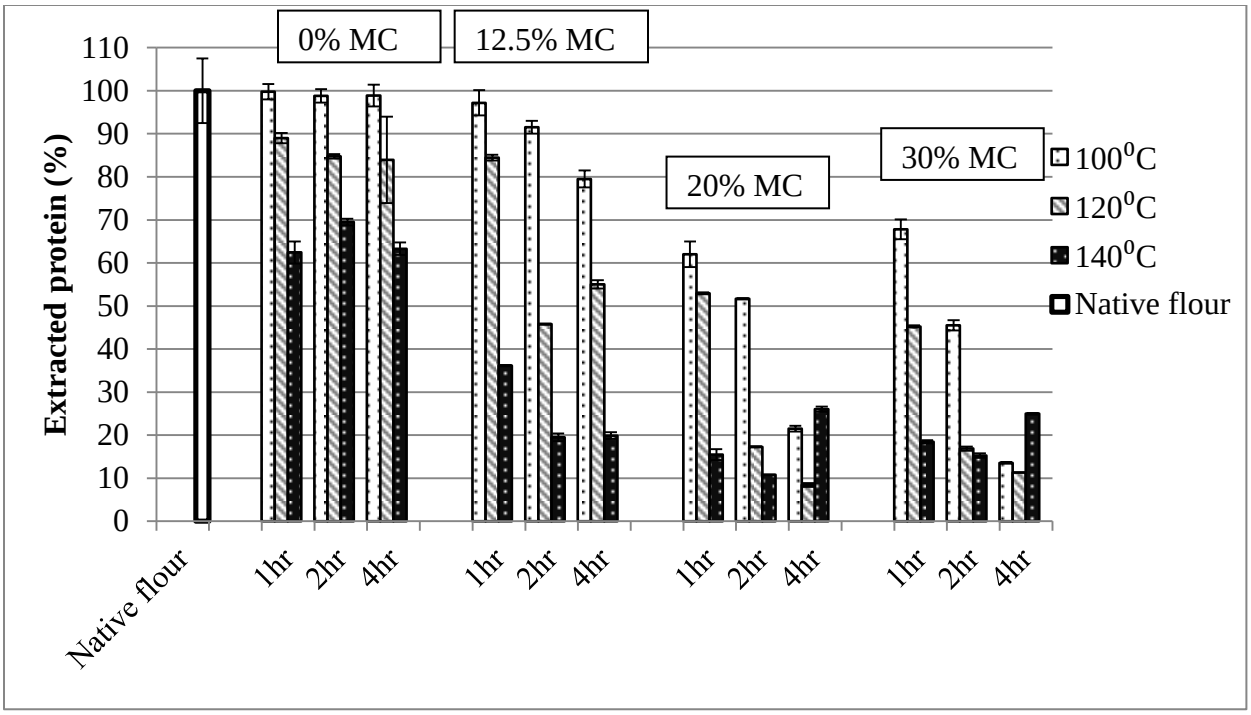


Figure 1.12. Protein extractability of sorghum flour after heat treatments at different times, temperatures, and MCs

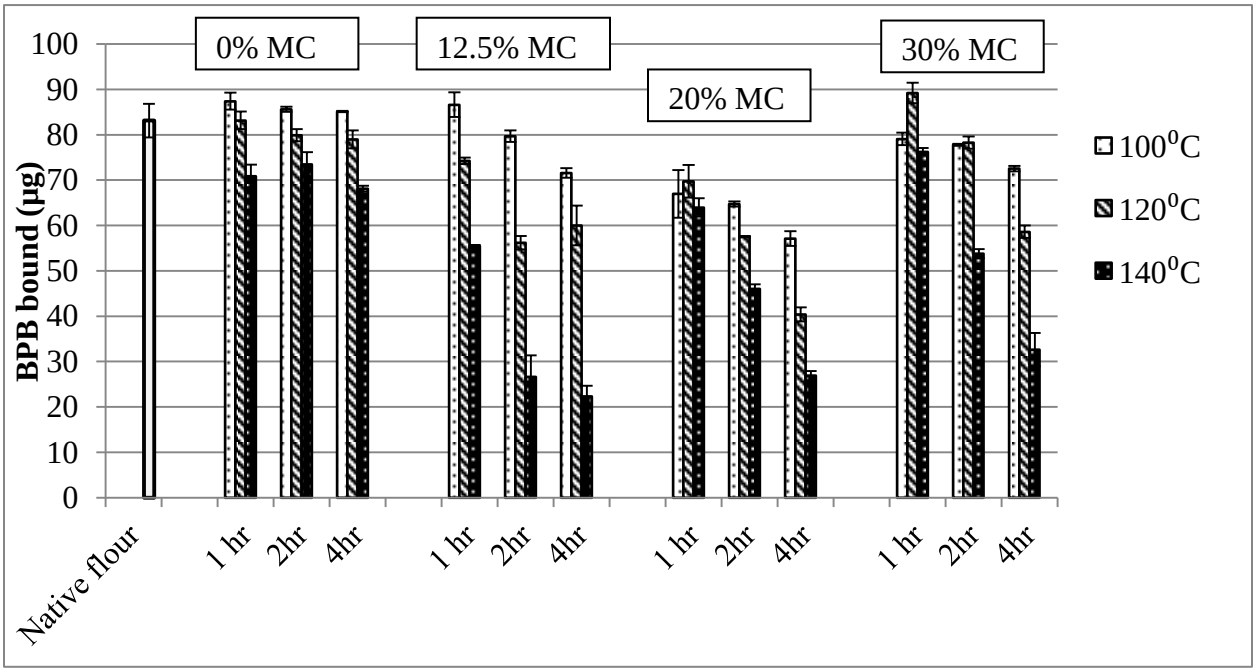


Figure 1.13. Surface protein hydrophobicity of sorghum flour before (native) and after heat treatments at different times, temperatures, and MCs

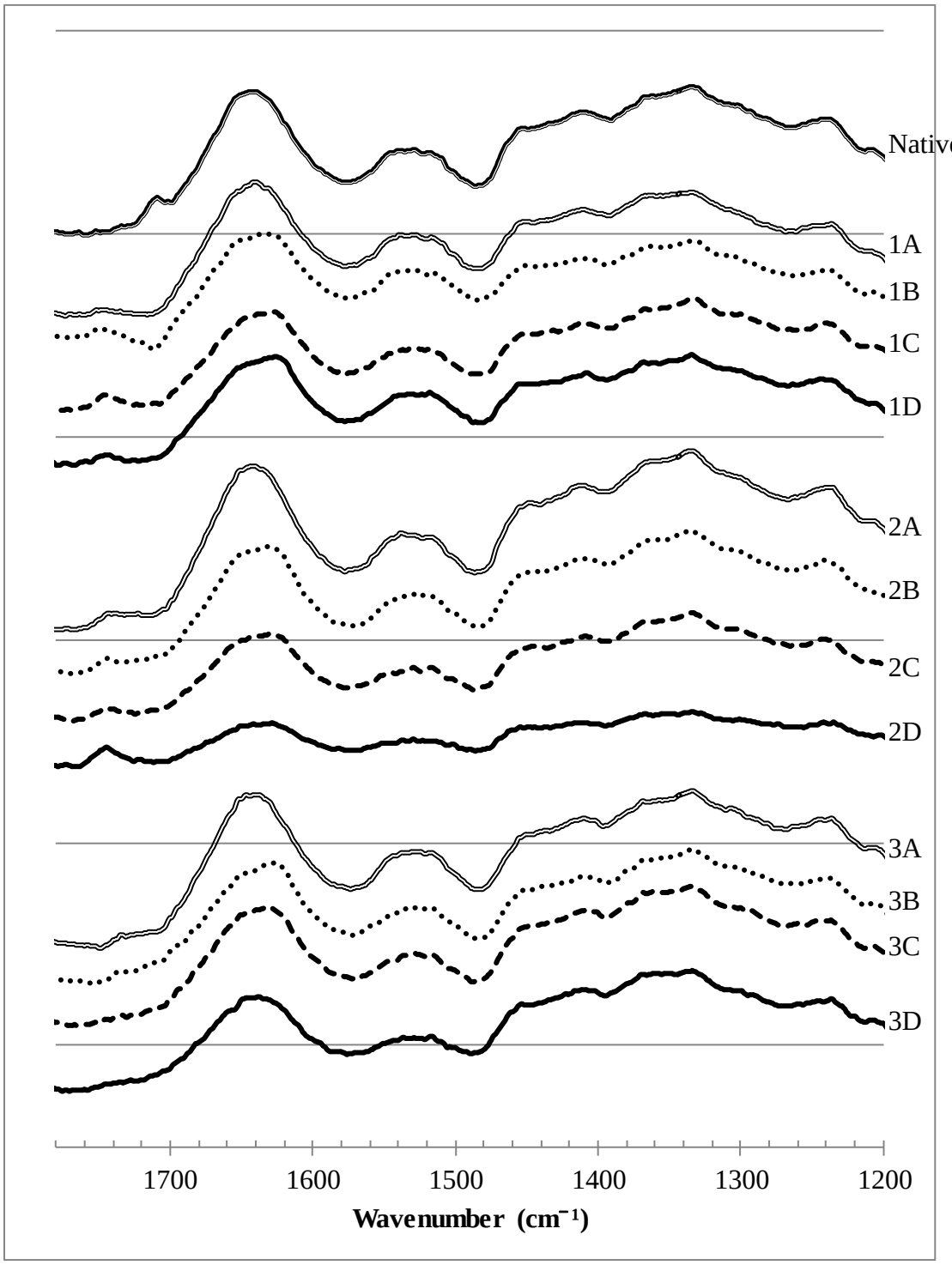


Figure 1.14. Fourier transform infrared spectra of the sorghum flour before (native) and after heat treatments for 4 h at different temperatures (1-100°C, 2-120°C, and 3-140°C) and MC (A-0%, B-12.5%, C-20%, and D-30%)

Chapter 2 - Starch digestibility of sorghum flour with heat treatment and pepsin treatment

Introduction

Resistant starch (RS) is defined as the starch that is not digested and absorbed in the small intestine (Englyst, Kingman, & Cummings, 1992). Because of the health benefits of dietary fiber in general and RS in particular, methods of testing RS and dietary fiber have been developed and improved to meet the need for testing and labeling amounts of dietary fiber in food. Testing methods for RS content were first developed by Berry (1986), and Englyst et al. (1992). These methods utilize *in vitro* digestion of starch using α-amylase and amyloglucosidase at temperatures close to that of the human body (37 °C); during testing, the digested starch is measured based on amount of glucose released, and RS content is calculated based on the difference between digested starch and total starch. Newer methods for measuring RS, including AOAC 985.29 (Prosky, Asp, Schweizer, Devries, & Furda, 1992) and AOAC 991.43 (Lee, Prosky, & Devries, 1992), employ thermally stable α-amylase to digest starch at boiling temperatures. In these methods, after starch digestion, samples are treated with protease and amyloglucosidase to remove protein and digestible starch, and RS is precipitated, filtered, dried, and corrected for protein and ash content. The most recent method (AOAC 2009.01), the integrated total dietary fiber (TDF) method, is a new procedure for RS testing that uses α-amylase and amyloglucosidase at 37°C for 16 h (B. V. McCleary et al., 2012). After digestion, the samples after digestion are treated with protease, precipitated with ethanol, filtered, washed, dried, and corrected for protein and ash content. This method can measure both high molecular weight dietary fiber (HMWDF) and low molecular weight soluble dietary fiber (LMWDF) by drying and weighing samples after α-amylase/amyloglucosidase digestion, and by liquid chromatography (LC), respectively.

RS content of the same sample can vary from method to method because of the differences in techniques to digest and measure starch (Maningat, Seib, & Bassi, 2013). For example, evaluating cross-linked wheat starch with the integrated TDF method (AOAC2009.01) results in low RS content (23.9%) compared with Englyst method (81.7%) (Shukri, Seib, Maningat, & Shi, 2013). In addition, starch digestion with both α-amylase and amyloglucosidase versus digestion with only α-amylase can result in different RS content in the same maize starch

sample (Brewer, Cai, & Shi, 2012). Which method provides a similar in-*vitro* starch digestion most similar to the human digestive system is at question.

Sorghum is more complicated to test for RS content than other grains. Its low protein and starch digestibility is unique among cereals. By testing with Englyst method, RS content of sorghum flour is high without efficient protease treatment (Xu, 2008). In fact, sorghum protein may act as a barrier to protect sorghum starch from enzymatic hydrolysis. The removal of sorghum protein by protease pretreatment helps expose the starch to digestive enzymes and is necessary to produce accurate results. A modified Englyst method with a longer time of protease treatment (2 h instead of 30 min) and different pH (2.0 instead of 1.3) was previously used to determine RS content of sorghum flour (Xu, 2008). The integrated TDF method, however, does not include a pepsin pretreatment step. Heat treatment was successful in increasing RS content of sorghum flour when evaluated by Englyst method (Chapter 1). The objectives of this chapter were to evaluate starch digestion of sorghum flour with and without heat treatment, and to investigate if a pepsin pretreatment step is needed in the integrated TDF method for testing RS content of sorghum flour.

Materials and methods

Materials

White sorghum flour (F1000) obtained from the USDA-ARS (Manhattan, KS) was used for RS content testing with and without pepsin treatment. White sorghum flour obtained from ADM (Archer Daniels Midland Company, Decatur, IL) before and after heat treatment was also used.

Englyst method

RS was measured by a modified *in vitro* Englyst method (Englyst et al., 1992). Flour samples (0.6 g) and guar gum (50 mg) were placed in a centrifuge tube (45 ml). Freshly prepared pepsin solution (50 mg pepsin in 10 ml 0.01M HCl) was added to the tube, and the mixture was incubated for 2 h at 37°C. Sodium acetate solution (0.25M) was added to the mixture to stop the digestion. Pancreatic/amyloglucosidase mixture (5 ml) and glass beads were added to the sample tube for starch digestion. The tube was incubated in a shaking water bath at 37°C and 90 strokes/min. At 20 and 120 min, an aliquot of 0.25 ml sample was pipeted into 10

ml of 66% ethanol. The glucose released at each interval was determined using the glucose oxidase/peroxidase method (Megazyme International Ireland, Co., Wicklow, Ireland) and was converted to percentage of starch hydrolyzed by multiplying by 0.9. Starch digested at 20 min was defined as RDS, starch digested between 20 and 120 min was defined as SDS, and starch not digested after 120 min of incubation was defined as RS.

Integrated total dietary fiber method

RS content of sorghum four was determined as the HMWDF fraction using Megazyme integrated total dietary fiber kit (Megazyme International). Sorghum flour (1.0g) was placed in a 250-ml bottle and wetted with 1ml of ethanol, followed by an addition of 40 ml α-amylase and amyloglucosidase in maleate buffer (50 mM, pH6.0 with 2 mM calcium chloride and 0.02% sodium azide). Samples were incubated with stirring at 37^{o}C for 16 h. After incubation, sample bottles were transferred to a boiling water bath for 10 min to deactivate enzymes. The bottles were placed into a water bath at 60 ^{o}C before 0.1 ml of protease was added to each sample for protein removal, and samples were incubated for 1 h at 60 ^{o}C. Four ml of acetic acid (2M) was added to the sample to decrease pH to 4.3 before 192 ml of 95 % ethanol (preheated to 60 ^{o}C) was added in to each sample to precipitate the undigested starch. The samples were filtered and washed with 78% ethanol twice then with acetone before being dried, weighed, and corrected with protein and ash content for calculating HMWDF or RS value (McCleary et al., 2012).

Modified integrated TDF method with protease pre-treatment

Sorghum flour was tested for RS content using a modified integrated TDF method with a protease pretreatment step for protein removal and glucose determination after starch digestion. Sorghum flour (1.0g) was subjected to protein pretreatment with 35ml pepsin (1.5mg/ml) phosphate buffer (pH 2.0) at 37 ^{o}C for 2 h prior to starch digestion. After the pepsin treatment, pH was increased to 6.0 by adding two ml of sodium hydroxide (2M). The samples were centrifuged, and supernatant was decanted. The residue was wetted with 1ml of ethanol followed by the addition of α-amylase and amyloglucosidase in 40 ml of maleate buffer (50 mM, pH6.0 with 2 mM calcium chloride and 0.02% sodium azide). Samples were incubated with stirring at 37 ^{o}C for 16 h. Aliquots (0.25 ml) were obtained from each sample after 2, 4, and 8 h of incubation and transferred to 15 ml centrifuge tubes each containing10 ml of 66.6% ethanol.

Glucose content of each sample was determined by the Megazyme D-glucose assay kit (Megazyme International).

Modified integrated TDF method with protease pretreatment and without addition of amyloglucosidase

Sorghum flour was subjected to a modified integrated TDF method with protease pretreatment, α-amylase starch digestion, and glucose determination after starch digestion. This method was similar to the previous modified ITDF method, but only α-amylase was used during the 16 h period of starch digestion in maleate buffer. An aliquot (0.5 ml) of each sample after α-amylase digestion was added to a 15-ml centrifuge tube with 10 ml of 66.6% ethanol. The tube was centrifuged, and 5ml of supernatant was transferred to a new 15ml tube with 10mg of amyloglucosidase to completely hydrolyze the α-amylase-digested-starch fraction into glucose. Glucose content of each sample was determined using the Megazyme D-glucose assay kit (Megazyme International).

Statistical analysis

Experiments on sorghum flour before and after heat treatments were done at least in duplicate. Collected data were analyzed by the analysis of variance (ANOVA) procedure with Duncan's multiple range test using SAS version 9.2 (SAS Institute Inc., Cary, NC). Mean values and standard deviations were reported.

Results and discussion

RS content of sorghum flour before and after heat treatment

Modified Englyst method

Sorghum flour after heat treatment had increased RS content compared with the native sorghum flour (Table 2.1). The increase in RS content was explained in the previous chapter, most likely because of the protein matrix outside the starch granule, or starch-protein interaction during heat treatment.

Integrated TDF method

A significant difference was found between RS content of sorghum flour with and without heat treatment, although the value differed only by 1% (Table 2.2). The RS content of normal sorghum flour was low with both the Englyst method (5.6%) and integrated TDF method (3.9%). For sorghum flour treated at 140^{o}C, 12.5 % MC, and 4h, RS content measured by the Englyst method (24.5%) was substantially higher than the integrated TDF method (4.9%). This finding agreed with a previous study by that compared RS content of cross-linked wheat starches tested with both methods (Shukri et al., 2013); due to long period of enzyme digestion (16 h) with both α-amylase and amyloglucosidase, 76.1% of the cross-linked wheat starch is digested (RS content of 23.9%)

RS content of sorghum flour after α-amylase and amyloglucosidase digestion at different time periods (2, 4, and 8 h)

Glucose determination was used in this method to estimate the amount of starch digested after pepsin pretreatment and starch digestion with both α-amylase and amyloglucosidase for different periods of time. Significantly lower starch digestion (higher RS content) was observed for sorghum flour with heat treatment than for native sorghum flour (Table 2.3). The heat treatment successfully increased starch resistant to enzymatic digestion. The differences in rate of starch digestion were more significant at shorter periods (2, 4, and 8 h) compared with the extensive 16-hour period where most of the starch was digested.

RS content of sorghum flour after α-amylase digestion for 16 h

When only α-amylase was used during starch digestion, a high level of RS content was obtained after 16 h of starch digestion (Table 2.4). A significantly higher RS content was observed for heat-treated sorghum starch (61.6%) than for native flour (45.0%). This result confirmed the resistance of heat-treated sorghum flour to starch enzyme digestion, especially to α-amylase.

Effects of pepsin treatments on RS content

Modified Englyst method

Table 2.5 shows lower RS content of sorghum flour with optimum pepsin treatment (pH2.0 for 2 h). With pepsin treatment, sorghum protein was removed, and starch was more exposed for enzyme digestion and which resulted in a lower RS level. This result agreed with a

previous study (Choi, Woo, Ko, & Moon, 2008) on the effects of protein removal using pepsin and bisulfite treatments on starch digestibility. The previous study found that higher starch digestibility of sorghum flour was obtained after pepsin and bisulfate treatments. Xu (2008) also reported similar results on digestibility of sorghum flour under different conditions of pepsin treatments.

Integrated TDF method

Results in Table 2.2 show no significant differences in RS content of sorghum flour with and without pepsin treatment before starch digestion and measurement by the integrated TDF method. These results are opposite those from the Englyst method. The protein matrix in sorghum flour protects starch granules from enzymatic digestion of starch. With long hour of starch digestion with continuous shaking, enzymes in the integrated TDF method with protease activity had overcome the protein matrix barrier and completely digested the sorghum starch. Protease activity (trypsin, 50mU/mg; chymotrypsin, 325mU/mg) of pancreatic α-amylase was reported by McCleary and Monaghan (2002). The integrated TDF method has been known to underestimate the RS content of different samples (Shukri et al., 2013). RS contents of samples measured by the Englyst methods (Englyst et al., 1992) are higher than those measured by the integrated TDF method (McCleary, 2007) for high amylose corn starch, raw potato starch, and corn flakes, and cross-linked phosphorylated wheat starch (Fibersym)(Shukri et al., 2013) due to the extensive period of starch digestion (16 h) with both α-amylase and amyloglucosidase at 37 ^{o}C.

RS content of sorghum flour after α-amylase and amyloglucosidase digestion at different time periods (2, 4, and 8 h)

Glucose determination was used in this method to estimate the amount of starch digested after pepsin pretreatment and starch digestion with both α-amylase and amyloglucosidase. Results in Table 2.6 show no significant differences in the RS content of sorghum flour with and without pepsin treatments after different time of starch digestion. Again, with the harsh conditions of continuous shaking for extended periods of time in the low viscosity buffer, starch enzymes with protease activity seemed to overcome the protein matrix barrier and digest sorghum starch regardless of pepsin pretreatment. These results were in agreement with

McCleary and Monaghan (McCleary & Monaghan, 2002), who reported that contaminated protease in the α-amylase can digest protein during starch digestion.

RS content of sorghum flour after α-amylase digestion for 16 hr

When only α-amylase was used during starch digestion after pepsin treatment, higher levels of RS was obtained after 16 h of starch digestion (Table 2.7).However, there is no differences in RS content with and without pepsin pretreatment. This result further confirmed that pepsin pretreatment is not needed for testing RS content of sorghum flour. The sudden decrease in starch digestibility with pretreatment showed the incompatible of the pretreatment with α-amylase activity. With phosphate and sodium ion left over after pretreatment that can partially inhibit α-amylase digestion, amount of starch digested was decreased.

Conclusions

Different modified integrated TDF methods were used to evaluate starch digestibility of sorghum flour with and without pepsin treatment, and with and without heat treatment. Heat treatment of sorghum flour decreased starch digestibility when the Englyst method and modified integrated TDF methods were used. Pepsin treatment, however, affected starch digestibility of sorghum flour only with the Englyst method. These results suggest protease activity of starch enzymes in the integrated TDF method, and demonstrate the effects of heat treatment on the starch digestibility of sorghum flour.

References

Berry, C. (1986). Resistant starch - formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. *Journal of Cereal Science, 4*(4), 301-314.

Brewer, L. R., Cai, L., & Shi, Y. (2012). Mechanism and enzymatic contribution to in vitro test method of digestion for maize starches differing in amylose content. *Journal of Agricultural and Food Chemistry, 60*(17), 4379-4387.

Choi, S. J., Woo, H. D., Ko, S. H., & Moon, T. W. (2008). Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfite on in vitro digestibility of waxy sorghum flour. *Cereal Chemistry, 85*(1), 65-69.

Englyst, H., Kingman, S., & Cummings, J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, S33-S50.

Lee, S. C., Prosky, L., & Devries, J. W. (1992). Determination of total, soluble, and insoluble dietary fiber in foods - enzymatic gravimetric method, mes-tris buffer - collaborative study. *Journal of AOAC International, 75*(3), 395-416.

Maningat, C., Seib, P., & Bassi, S. (2013). Dietary fiber content of cross-linked phosphorylated resistant starch (RS4) determined by the prosky and McCleary methods part II. comparison of assay data. *Cereal Foods World, 58*(5), 252-253-263.

McCleary, B. V., & Monaghan, D. A. (2002). Measurement of resistant starch. *Journal of AOAC International, 85*(3), 665-675.

McCleary, B. V. (2007). An integrated procedure for the measurement of total dietary fibre (including resistant starch), non-digestible oligosaccharides and available carbohydrates. *Analytical and Bioanalytical Chemistry, 389*(1), 291-308.

McCleary, B. V., DeVries, J. W., Rader, J. I., Cohen, G., Prosky, L., Mugford, D. C., . . . Okuma, K. (2012). Determination of insoluble, soluble, and total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: Collaborative study. *Journal of AOAC International, 95*(3), 824-844. doi:10.5740/jaoacint.CS2011_25

Prosky, L., Asp, N. G., Schweizer, T. F., Devries, J. W., & Furda, I. (1992). Determination of insoluble and soluble dietary fiber in foods and food-products - collaborative study. *Journal of AOAC International, 75*(2), 360-367.

Shukri, R., Seib, P. A., Maningat, C. C., & Shi, Y. (2013). In vitro enzymatic testing method and digestion mechanism of Cross-linked wheat starch. *Resistant Starch Sources, Applications and Health Benefits, ,* 145-166.

Xu, X. (2008). *In Vitro Digestibility of Starch in Sorghum Differing in Endosperm Hardness and Flour Particle Size,*

Tables

Table 2.1. Resistant starch (RS) content (mean ± SD %) on starch basis of ADM sorghum flour and heat treated (14°C, 12.5% MC, 4 hr) ADM sorghum flour (Englyst method with optimum pepsin treatment)

Treatment	RS content (%)
No heat treatment	5.6±2.6[b]
With heat treatment	24.5±1.1[a]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 2.2. Resistant starch (RS) content (% starch basis) of ADM sorghum flour with heat treatment (12.5% MC, 140°C, 4hr) and pepsin treatment (Integrated TDF method)

Treatment	RS content (%)
No pepsin treatment	3.9±0.4[b]
With pepsin treatment	4.4±0.1[a,b]
Heat treated sorghum flour with no pepsin treatment	4.9±0.1[a]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 2.3. Resistant starch (RS) (mean ± SD % starch basis) of ADM sorghum flour and heat treated (140°C, 12.5% MC, 4 hr) ADM sorghum flour after different time of starch digestion with α-amylase and amyloglucosidase (Modified Integrated TDF method with glucose content measurement after 2, 4, and 8hr of starch digestion)

Time	No heat treatment	With heat treatment
2h	58.5±2.2[b]	64.4±1.0[a]
4h	33.5±2.5[b]	41.9±1.5[a]
8h	5.7±2.5[b]	13.3±2.2[a]

[A] Values with the same letter in the same row are not significantly different ($p<0.05$)

Table 2.4. Resistant starch (RS) content (% starch basis) of ADM sorghum flour and heat treated ADM sorghum flour (140°C, 12.5% MC, 4 h) after 16 h of starch digestion (modified integrated TDF method with phosphate buffer pepsin treatment and α-amylase only)

Treatment	RS content (%)
No heat treatment	45.0±0.7[b]
With heat treatment	61.6±0.6[a]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 2.5. Resistant starch (RS) content (% on starch basis) of F1000 (Englyst method)

Treatment	RS content (%)
With optimum pepsin treatment	8.9±2.3[b]
No optimum pepsin treatment	16.9±1.7[a]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 2.6. Resistant starch (RS) (mean ± SD % starch basis) of F1000 sorghum flour after different time of starch digestion with α-amylase and amyloglucosidase (modified integrated TDF method with glucose measurement after 2, 4, and 8 h of starch digestion)

Time	No pepsin treatment	With pepsin treatment
2h	63.7±2.6[a]	64.6±1.9[a]
4h	34.4±1.8[a]	34.5±2.5[a]
8hr	-0.5±0.9[a]	4.1±3.5[a]

[A] Values with the same letter in the same row are not significantly different ($p<0.05$)

Table 2.7. Resistant starch content (% starch basis) of F1000 sorghum flour after 16 h of α-amylase digestion (Modified integrated TDF method with HCl pepsin treatment and α-amylase only)

Treatment	RS content (%)
No pepsin treatment	40.6±3.2[a]
With pepsin treatment	36.1±2.5[a]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Chapter 3 - Evaluation of sorghum flour in tortillas

Introduction

In 2012, the United States produced 246,932 bushels (6.2 million metric tons) of sorghum, and US sorghum production is projected to increase by 60% in 2013 (Improved US sorghum production lifts export goals: Strong marketing a key. 2013). The US is also the second largest sorghum producer in the world and the first largest sorghum exporter. While most of the sorghum produced in the US is exported (41.9%) and used for feed (35.4%), 22.7% sorghum produced is used for food and industrial purposes. According the US grain council, 12% of sorghum produced in US is utilized in ethanol production. That leaves about 10% of sorghum for food products (Sorghum.2010).

Sorghum is a staple food in semi-arid area of Asia, Africa and Central America and is used in different food products including porridges, couscous, flatbreads, syrup, and fermented beverages (Sorghum.2010). In the US, the use of sorghum flour is limited in foods. However, there is an increasing demand for sorghum food products since sorghum is gluten-free and can be used for people with celiac disease.

To evaluate our heat treated sorghum flour compared with normal sorghum flour in a food application, sorghum tortillas were used. A sorghum tortilla formula had been developed by (Fernholz, 2008) that used mild cooking conditions (6 seconds of pressing at 230^{o}F and 30 seconds of griddle cooking at 350^{o}F). The low heat cooking condition may help retain resistant starch content of the sorghum flour without completely gelatinizing the starch. However, this sorghum tortilla formula did not produce comparable tortillas to wheat tortillas.

Many improvements can be made to increase sorghum tortillas quality. In above tortilla formula, xanthan gum is used for texture functionality. Tortilla texture can be improved by increase amount of xanthan gum or using xanthan gum in combination with locust bean gum to form a synergistic gelation (Copetti, Grassi, Lapasin, & Pricl, 1997).

The objectives of the work were to improve sorghum tortilla formulation and evaluate the use of sorghum flour with and without heat treatment for the production of wheat-free tortillas. The use of sorghum flour with and without heat treatment in replacement for wheat flour in flour tortilla formula was also evaluated.

Materials and methods

Sorghum tortillas preparation

Sorghum tortillas were prepared according to formula (Table 3.1) and procedure from Fernholz (2008) with modifications to produce smaller batches of dough (enough to make 3 tortillas)

Ingredients including sorghum flour, sugar, shortening and glycerin were mixed for one minute using a pin mixer (Figure 3.1.a). Other dry ingredients were mixed in over the next 30 seconds. Water was added and the mixture was mixed for another minute. After that, the bowl and pin were scraped and mixed for two min. The mixed dough was divided into 25g dough balls and placed in bowl with a lid on. Dough balls (Figure 3.1.b) were used for tortillas cooking within 30 min. For cooking of tortillas, the dough ball was pressed in-between two pieces of parchment paper by the pre-heated presser (45^{o}C) with the thickness setting of 1.5mm. The pressed dough was transferred onto the griddle (191^{o}C) and cooked for 45 seconds per side (Figure 3.1.c). Sorghum tortillas after cooking were allowed to cool on rack (Figure 3.1.d) and placed in Ziploc bags. Double the amount of xanthan gum was tested to improve texture of the tortillas. Locust bean gum (LBG) was also used to replace 50% of xanthan gum in this formula.

Wheat tortillas preparation

Wheat tortillas were prepared according to formula (Table 3.2). 100%, 75% and 60% wheat flour were used to made tortillas with amounts of 0%, 25%, and 40% sorghum flour, respectively.

The mixograph was used to determine water absorption and mixing time of wheat flour and blends of wheat and sorghum flour. Minor ingredients including salt, calcium propionate, potassium sorbate, SALP, soda, and fumaric acid were weighted and mix together to create a blend of dry ingredients. To make three tortillas, 52.33 g of wheat flour, 3.14 g of shortening, and 2.03 g of dry ingredient blend were mixed for 3.5 min (optimum mixing time from the mixograph plus 30 seconds) with 27.5 ml (optimum absorbance less 10%) of water in a pin mixer. After mixing, the dough was divided into 25g dough balls and rested for 20 min. Dough balls were pressed using a pre-heated tortilla presser at 325^{o}F for 3 seconds, and was transferred to a hot griddle (375^{o}F). Tortillas were cooked on the hot griddle for 30 seconds on one side, 20

seconds on the second side, and the first side again for 10 seconds. Tortillas were cooled on rack and kept in Ziploc bag until measurements.

Different amount of sorghum flour were used to replace wheat flour in flour tortilla formula. (Torres, Ramirez-Wong, Serna-Saldivar, & Rooney, 1993) has in studied on the effect of sorghum flour replacement in wheat tortillas and found that quality of wheat tortillas are still acceptable with up to 25% of sorghum flour added. In this study, 25% and 40% of sorghum flour with and without heat treatment were added into wheat tortillas.

Tortilla testing

Weight, diameter, and thickness

Tortillas were weighed individually. Diameter was measured using three measurements by turning the tortillas 45 degree for the second and third measurement. To measure tortillas' thickness, a digital caliper was used.

Stretchability/flexibility

Tortilla texture was tested with the TA-TXT2 texture analyzer. Each tortilla was fixed on the TA-108 Tortilla/Film fixture. A TA-18B probe in ball shape with diameter of one inch was used for this puncture test (Figure 3.2) following the settings as described by Fernholz (2008): pre-test speed of 6.0 mm/s, test speed of 1.70 mm/s, post-test speed 10mm/s with the distance of 30mm and force of 20.0 g.

Statistical analysis

Experiments on sorghum flour before and after heat treatments were done at least in duplicate. Collected data were analyzed by analysis of variance (ANOVA) procedure with Duncan's multiple range test using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Mean values and standard deviation were reported.

Results and discussion

Tortilla weight, diameter, and thickness

Compared with wheat tortillas, sorghum tortillas were thinner but larger in diameter (Table 3.3 & Figure 3.3). Sorghum tortillas made with heat treated sorghum flour (20% MC, 4 hrs, at 100^{o}C) were larger and thinner because of differences in pasting properties of heat treated flour and normal sorghum flour. Adjusting the thickness setting of the tortilla press may help improve the thickness and diameter of the tortillas made with heat treated flour.

Weight, diameter, and thickness of wheat tortillas with 25% and 40% sorghum flour replacement were similar to the measurements of 100% wheat tortillas (Table 3.4 and Figure 3.4). Replacement of sorghum flour to wheat flour in wheat tortilla formula could be a good alternative to making 100% sorghum tortillas. Also, there was no significant different between the tortillas made with normal sorghum flour and heat treated sorghum flour.

Tortilla texture

Tortillas made with 100% sorghum flour were significantly weaker in texture with lower force needed to puncture the tortillas compared with wheat tortillas made with the same formula (Table 3.5). Increased amount of xanthan gum can improve texture of the tortillas; however, the stretchability of sorghum tortillas with high amount of xanthan gum was not comparable to that of wheat tortillas. Stretchability of sorghum tortillas were also decreased after aging, making the tortillas easier to break. Starch retrogradation during aging could cause the tortillas texture to be more brittle.

Tortillas made with wheat tortillas formula were significantly stronger in texture than the wheat tortillas made with sorghum flour formula (Table 3.6). Tortillas made with 25% and 40% sorghum flour had significant lower force and distance needed for the probe to break the tortillas compared with the control samples. With increasing amount of sorghum flour replacement to wheat flour, stretchability of the tortillas decreased. However, the different was not significant between 25% and 40% sorghum flour replacement for the measure on the same day of tortilla making. After storage for 7 days, the force and distance needed to break the tortillas decreased significantly. Also there was a significant decrease in tortilla texture with increase in sorghum flour content. Between the normal sorghum flour and heat treated sorghum flour, there was no significant difference in texture of tortillas made with different sorghum flour.

Conclusion

The use of sorghum flour with and without heat treatment in different tortillas formulation was evaluated. We found that sorghum flour was not suitable to completely replace wheat flour even with the use of different amounts and combinations of gums. Since the texture of flour tortillas is very important in the final quality of tortillas, and gluten is the main factor in texture quality of tortillas, replacing wheat flour with sorghum flour was very challenging. The use of sorghum flour in replacement of wheat flour in other baked products such as cookies (where gluten texture is not as important as in tortillas) should produce acceptable products with sorghum flour.

References

Copetti, G., Grassi, M., Lapasin, R., & Pricl, S. (1997). Synergistic gelation of xanthan gum with locust bean gum: A rheological investigation. *Glycoconjugate Journal*, *14*(8), 951-961.

Fernholz, M. C. (2008). *Evaluation of Four Sorghum Hybrids through the Development of Sorghum Flour Tortillas*,

Improved US sorghum production lifts export goals: Strong marketing a key. (2013). Retrieved 10/09, 2013, from http://www.grains.org/index.php/2012-04-30-15-22-26/4289-improved-us-sorghum-production-lifts-export-goals-strong-marketing-a-key

Sorghum. (2010). Retrieved 10/09, 2013, from http://www.grains.org/index.php?option=com_content&view=article&id=74&Itemid=1

Torres, P., Ramirez-Wong, B., Serna-Saldivar, S., & Rooney, L. (1993). Effect of sorghum flour addition on the characteristics of wheat flour tortillas. *Cereal Chemistry, 70*(1), 8-13.

Tables and Figures

Table 3.1. Sorghum tortillas formula

Ingredient	Baker's Percentage	Amount, g
Sorghum flour	100.00	80.0
Salt	2.50	2.0
Xanthan gum	1.00	0.8
Baking powder	0.75	0.6
Glycerin	6.75	5.4
Vegetable shortening	11.50	9.2
Citric acid	0.50	0.4
Sugar (granulated)	15.00	12.0
Monoglyceride (Dimodan PH300 K-A, Danisco)	2.00	1.6
Water	65.00	52.0
TOTAL		164.0

Table 3.2. Wheat tortillas formula

Ingredient	Flour weigh base (%)	Amount, g
Wheat flour	100.00	52.33
Shortening	6.00	3.14
Salt	1.50	0.78
Calcium propionate	0.50	0.26
Potassium sorbate	0.40	0.21
SALP	0.58	0.30
Soda	0.60	0.31
Fumaric Acid	0.24	0.13
Water	Optimum absorption less 10%	
TOTAL		≈80

Table 3.3. Weight, diameter and thickness of tortillas made with wheat flour (A), sorghum tortillas (B), sorghum tortillas with double amount of xanthan gum (C), sorghum tortillas with LBG and xanthan gum (D), sorghum tortillas with double amount of LBG and xanthan gum (E), sorghum tortillas with double amount of xanthan gum and heat treated sorghum flour (F).

Sample	Weight (g)	Diameter (cm)	Thickness (mm)
Wheat tortilla A	21.39±0.07[a]	11.24±0.28[c]	2.06±0.09[a]
Sorghum tortilla B	21.66±±0.08[a]	12.02±0.07[b]	1.66±0.04[b,c]
Sorghum tortilla C	21.57±0.14[a]	12.16±0.12[b]	1.69±0.06[b]
Sorghum tortilla D	20.83±0.12[b]	12.11±0.47[b]	1.52±0.05[c]
Sorghum tortilla E	21.18±0.24[a]	12.20±0.47[b]	1.57±0.10[c]
Sorghum tortilla F	20.03±0.25[c]	13.86±0.35[a]	1.29±0.11[d]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 3.4. Weight, diameter and thickness of wheat tortillas wheat tortillas made with 100% wheat flour (A), 75% wheat flour/25% sorghum flour (B), 75% wheat flour/25% heat treated sorghum flour (C), 60% wheat flour/40% sorghum flour (D), and 60% wheat flour/40% heat treated sorghum flour (E)

Sample	Weight (g)	Diameter (cm)	Thickness (mm)
A	21.87±0.06[a,b]	11.91±0.13[a]	2.46±0.04[a]
B	21.56±0.17[a,b]	11.87±0.32[a]	2.02±0.10[b]
C	21.52±0.26[b]	12.13±0.27[a]	1.91±0.08[b]
D	21.92±0.23[a]	11.99±0.30[a]	1.90±0.16[b]
E	21.60±0.19[a,b]	11.83±0.20[a]	1.89±0.11[b]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 3.5 Stretchability of tortillas made with wheat flour (A), sorghum tortillas (B), sorghum tortillas with double amount of xanthan gum (C), sorghum tortillas with LBG and xanthan gum (D), sorghum tortillas with double amount of LBG and xanthan gum (E), sorghum tortillas with double amount of xanthan gum and heat treated sorghum flour (F)

Sample	Day 1		Day 8	
	Force (g)	Distance (mm)	Force (g)	Distance (mm)
Wheat tortilla A	449.4±29.5[a]	13.3±0.6[a]	303.5±35.2[a]	9.0±1.1[a]
Sorghum tortilla B	108.8±3.8[b]	6.0±0.4[b]	84.0±3.0[b]	6.1±0.7[b]
Sorghum tortilla C	131.4±4.8[b]	6.2±0.4[b]	101.1±5.1[b]	4.4±0.4[b,c]
Sorghum tortilla D	90.4±4.0[b]	5.6±0.3[b]	101.8±7.0[b]	5.3±0.8[b,c]
Sorghum tortilla E	116.2±11.9[b]	6.0±0.1[b]	105.3±4.8[b,c]	4.8±0.6[c]
Sorghum tortilla F	108.4±8.8[b]	5.1±0.2[b]	62.1±11.3[c]	4.0±0.5[c]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

Table 3.6. Stretchability of wheat tortillas made with 100% wheat flour (A), 75% wheat flour/25% sorghum flour (B), 75% wheat flour/25% heat treated sorghum flour (C), 60% wheat flour/40% sorghum flour (D), and 60% wheat flour/40% heat treated sorghum flour (E). The heat treated sorghum flour was treated at 100°C with 20% MC for 4hr

Sample	Day 1		Day 8	
	Force (g)	Distance (mm)	Force (g)	Distance (mm)
A	1130.0±165.1[a]	23.0±2.9[a]	481.9±76.6[a]	12.4±0.4[a]
B	824.7±248.6[b]	17.7±2.5[b]	362.2±45.5[b]	9.9±0.8[b]
C	731.3±96.9[b]	17.1±1.4[b]	399.0±31.8[b]	9.5±0.8[b]
D	647.0±146.9[b]	15.3±2.0[b]	232.3±4.7[c]	7.8±0.5[c]
E	695.1±69.0[b]	16.6±0.5[b]	283.7±16.9[c]	8.3±0.2[c]

[A] Values with the same letter in the same column are not significantly different ($p<0.05$)

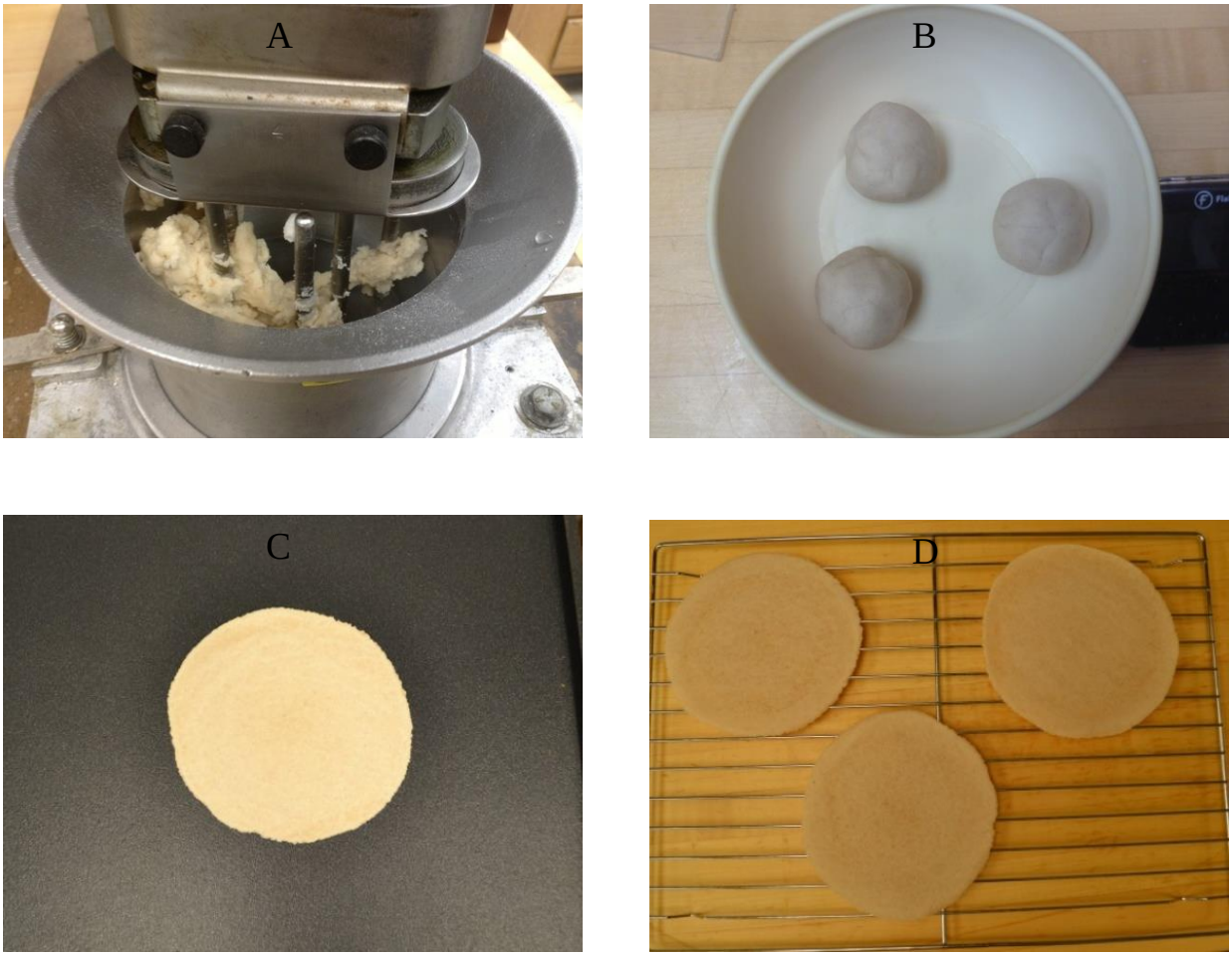


Figure 3.1. Tortillas preparation: mixing (A), dough balls (B), griddle cooking (C) and cooling (D)

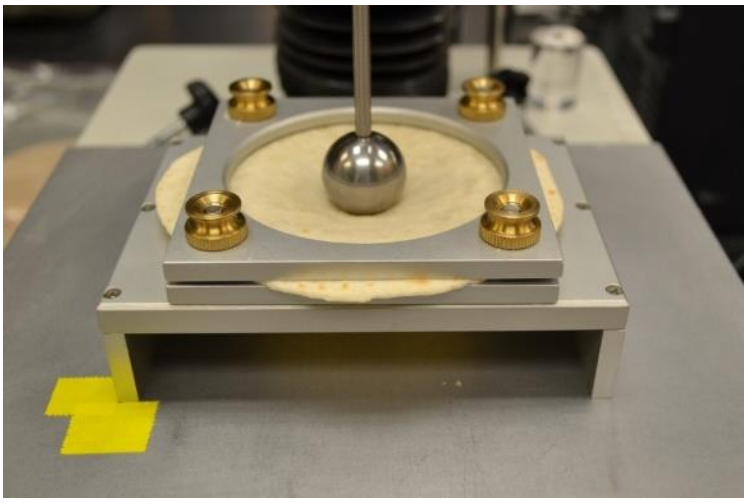


Figure 3.2. Texture analyzer for measuring stretchability/flexibility of tortillas

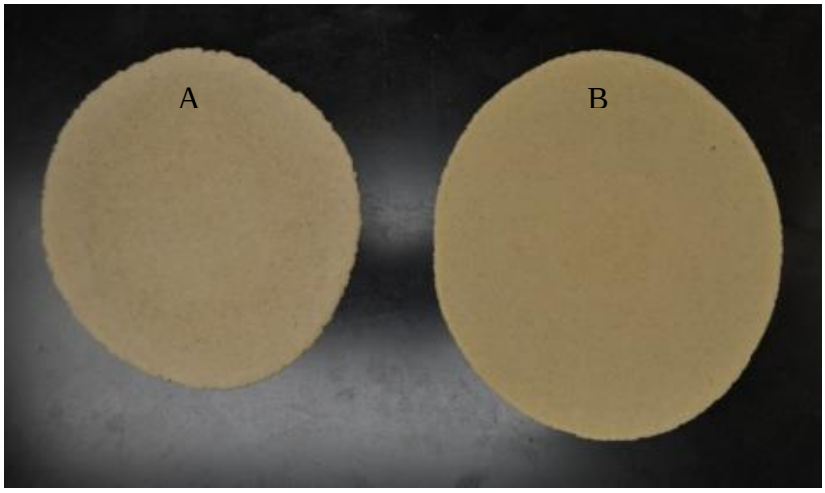


Figure 3.3. Tortillas made with normal sorghum flour (A) and heat treated sorghum flour (B)

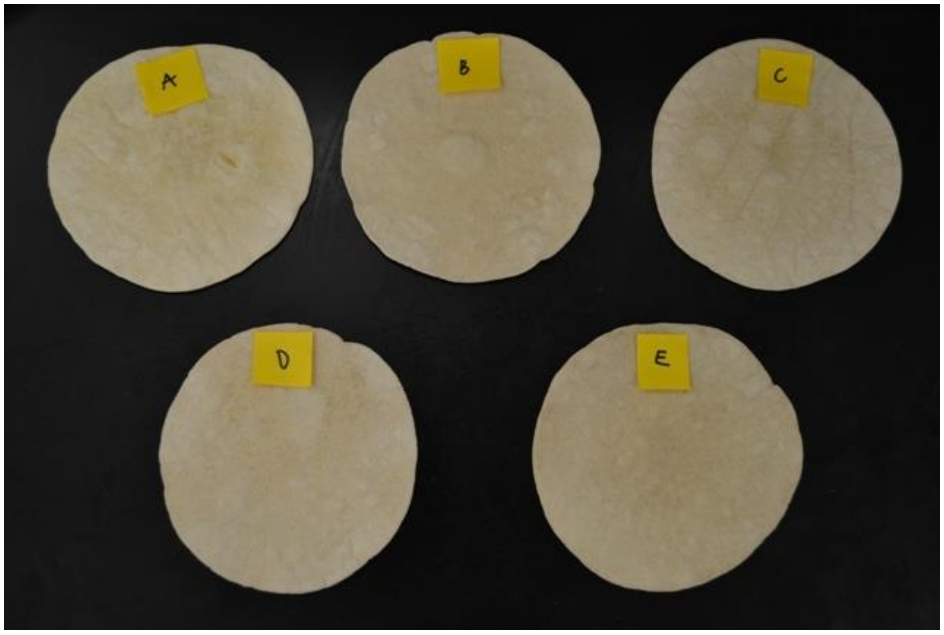


Figure 3.4. Tortillas made with wheat flour (A), 75% wheat flour and 25% sorghum flour (B), 75% wheat flour and 25% heat treated sorghum flour (C), 60% wheat flour and 40% flour(D), 60% wheat flour/40% heat treated sorghum flour (E).