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Ozone gas affects physical and chemical properties of wheat (*Triticum aestivum* L.) starch

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ABSTRACT

In this experiment, bread wheat flour and isolated wheat starch were treated with ozone gas (1,500 mg/kg at 2.5 L/min) for 45 min and 30 min, respectively. Starch was isolated from treated flour. Ozone treated starch and starch isolated from ozone treated flour had similar chemical and physical properties. Chemical analysis of starch isolates indicated depolymerization of high molecular weight amylopectins; with a subsequent increase in low molecular weight starch polymers as a result of starch hydrolysis. Ozone treatment resulted in elevated levels of carboxylic groups and decreased total carbohydrate content in amylopectin fractions. ^{1}H NMR results indicated formation of a keto group [(1→4)-3 keto] at the H-2 terminal (proton at C-2 position) and β-glucuronic acid at the H-1 terminal (proton at C-1 position). DSC transition temperatures and change in enthalpy were not affected by ozone treatment. Increased swelling power and RVA breakdown were observed in starch from ozone treated samples.

1. Introduction

Oxidized starches have industrial and food applications. Oxidized starches typically have low viscosity and high stability, film forming, and binding properties (Kuakpetoon & Wang, 2006). Oxidized starches are used as coating and sealing agents in confectionaries, as emulsifiers and dough conditioners in bread, and as binding agents in batter (Chattopadhyay, Singhal, & Kulkarni, 1997; Konoo, Ogawa, Mizuno, & Iso, 1996; Thomas & Atwell, 1999).

Oxidized starches are produced by reacting hydroxyl groups present on the amylose and amylopectins with oxidizing reagents under controlled temperature and pH (Wurzburg, 1986). Hypochlorite, sodium bromide, gaseous chlorine, calcium hypochlorite, hydroperoxide, potassium permangnate, ammonium persulfate, oxygen, and bromide are different types of chemical oxidizing agents used to oxidize starch (Muhrbeck & Eliasson, 1990; Wing, 1994).

Chemical agents used for oxidation of starches can create safety and environmental hazards (Chan, Bhat, & Karim, 2009), chemical waste problems, and leave undesirable residues in final products (An & King, 2009). The search for a more environmentally friendly method to oxidize starch has lead to interest in ozone gas. Ozone gas is a powerful oxidant (oxidation–reduction potential of 2.07 V) that has several advantages over traditional chemical oxidizing agents: (1) does not leave a residue, (2) can be generated on site, (3) requires no storage and subsequent disposal of chemical containers, and importantly (4) United States FDA granted generally recognized as safe (GRAS) status for the use of ozone as a disinfectant and sanitizer for foods "when used in accordance with good manufacturing practices" (Guzel-Seydim, Greene, & Seydim, 2004).

Oxidizing agents can oxidize hydroxyl groups present at C2, C3, and C6 positions on glucose molecule to carbonyl and carboxyl groups (BeMiller, 2007). Introduction of carboxyl and carbonyl groups increase swelling power and alter pasting properties of starch (Chan et al., 2009; Kuakpetoon & Wang, 2001). The increase in swelling power of ozone oxidized starches has been related to the introduction of hydrophilic carboxyl groups (Chan et al., 2009). The negative charges of carboxyl groups repel each other causing increased swelling of starch granules during heating in water. Chan et al. (2009) reported a positive correlation ($r = 0.63$, $P < 0.01$) between carboxyl content and swelling power of ozone oxidized corn (*Zea mays* L.) starch.

An and King (2009) reported that commercial rice (*Oryza sativa*) starch treated with ozone had similar pasting properties as starch oxidized with low concentrations of chemical oxidizing agents. Chan et al. (2009) reported significant decline in swelling power of ozone oxidized sago (*Metroxylon sago*) and tapioca (*Manihot esculenta*) starches but an increase in swelling power of ozone oxidized corn starch. Ozone treatment decreased the solubility of tapioca starch, increased solubility of sago starch, and had little effect on solubility of corn starch. It was reported that ozone treatment of soft wheat flour did not affect the transition temperature or enthalpy change of the flour samples (Chittrakorn, 2008). Sandhu, Manthey,

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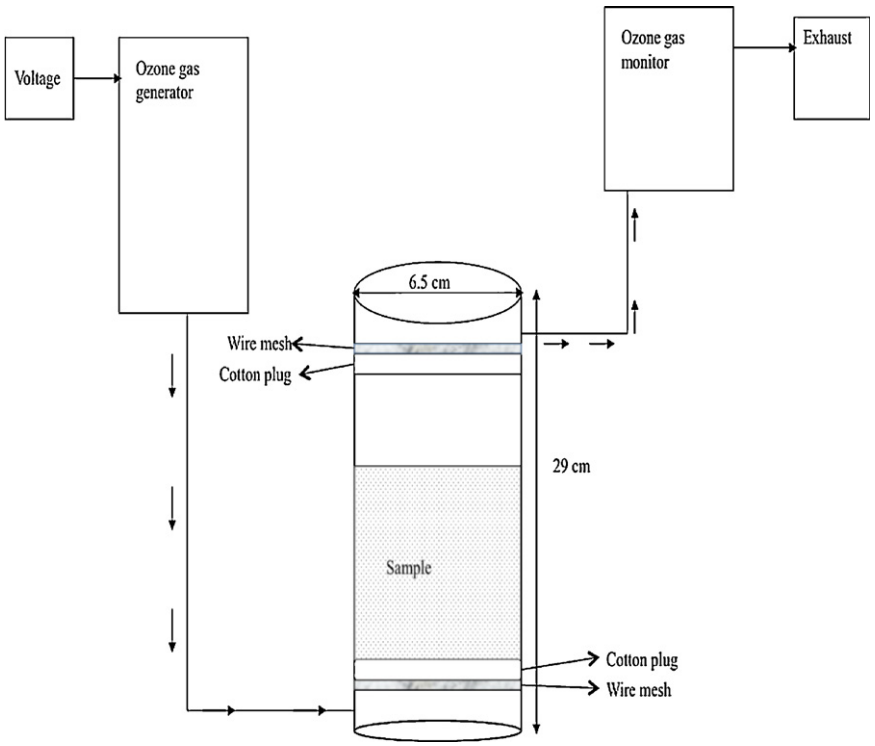

Fig. 1. Schematic representation of method used to treat sample with ozone gas.

and Simsek (2011), also reported an increase in peak viscosity, set back viscosity, and final viscosity of commercial wheat flour treated with ozone.

Most of the research has been done using ozone as an oxidant on isolated starch from corn, tapioca, rice, sago and waxy starch (An & King, 2009; Chan et al., 2009). However, limited research has been published using ozone as an oxidant on wheat flour or on wheat starch. Hence, this research was conducted to determine the effect of ozone treatment on wheat flour and on isolated wheat starch on the physicochemical properties of wheat starch.

2. Materials and methods

2.1. Starch isolation

Bread wheat flour used in this study was procured from three commercial sources. These flours were not bleached and did not contain malt or any other added ingredients. These commercial flours were treated as three replicates. Starch was isolated from these flour samples by the dough washing method described by Lu and Grant (1999). Starch was obtained by washing the flour (10 g) with sodium chloride solution (2%, w/w in distilled water) using a Glutomatic 2200 gluten washing system (Perten instruments, Springfield, IL). The isolated starch was washed with distilled water and centrifuged at $537 \times g$ for 15 min. Salt was removed from starch samples by dialysis. Starch samples were poured in a Spectra/Por dialysis membrane (MW CO: 1000 molecular weight) and membranes were sealed from both ends using clips. Sealed starch samples were placed in a large bucket of distilled water (4–5 °C) that was stirred at extremely slow speed. Distilled water was changed after every 8 h for 72 h. The purified starch was freeze-dried under vacuum using a freeze-dryer (model no: 12525; The Virtis Company, Inc. Gardiner, NY). Freeze-dried samples were grounded to fine particle size using a mortar and pestle and stored in plastic bags at 4–5 °C until used.

2.2. Ozone treatment

Flour and starch isolated from non-ozone-treated flour were treated with ozone gas using an ozone generator (OS-8C; Ozone Solutions, Inc.; Sioux Center, IA) equipped with an ozone monitor (model 450M, Advanced Pollution Instrumentation, Inc., San Diego, CA) (Fig. 1). An ozone gas destruct unit inside the monitor converted most of the ozone into oxygen before it was exhausted from the instrument. Sample (starch, 50 g and flour, 100 g) was placed in a cylindrical acrylic container (6.5 cm × 29.0 cm) having an inlet valve at one end and an outlet valve at the opposite end. Muslin cloth was used as a filter to stop the sample from flowing into the inlet and outlet pipes. Outlet valve was attached to the ozone monitor. Cylindrical acrylic container was one-quarter full with starch sample (50 g) and one-half-full with flour (100 g) sample. Samples were exposed to ozone gas (1,500 mg/kg, gas flow rate 2.5 L/min). Cylindrical acrylic container was rotated back and forth and sideways once every 5 min during treatment to promote uniform exposure of the sample to ozone.

Flour was exposed to ozone gas for 45 min and starch (50 g) was exposed to ozone gas for 30 min. The sample was considered fully treated when the concentration of ozone gas (1,500 mg/kg, gas flow rate 2.5 L/min) entering the cylinder was similar to the concentration of ozone gas (1,500 mg/kg, gas flow rate 2.5 L/min) leaving the cylinder. Preliminary experiments showed that starch and flour samples were fully treated with ozone gas (1,500 mg/kg) by 30 and 45 min, respectively. Ozone treated samples were placed in plastic bags, which were left open for 10 min before closing to allow residual ozone to dissipate. All samples were then stored at 4 °C until used.

2.3. Safety emphasis

Ozone primarily affects respiratory tract in humans, the air around the ozone equipment and work area was exhausted to the outside using an exhaust hood. Disposable gloves (Microflex®,

Microflex corp., Reno, NV) and nose masks (Dura-Mesh®, Airgas North Central, West Chicago, IL) were used while handling samples, and when working with ozone.

2.4. Amylose/amylopectin content

Determination of amylose and amylopectin in extracted starch samples was done using high pressure size exclusion column (HPSEC) chromatograph, according to a method developed by Grant, Ostenson, and Rayas-Duarte (2002). Starch (25 mg) was dissolved in 4.5 mL of 1 M KOH/urea solution and heated under nitrogen at 100 °C for 90 min (Morrison & Laignelet, 1983). The dissolved sample (1 mL) was neutralized with 1.0 M HCL and filtered through a 13 mm dia., 45 μm hydrophilic nylon syringe filter. The samples were analyzed at 40 °C and deionized, distilled water was used as an eluant. The sample was then analyzed with an Agilent 1200 series HPSEC (Agilent Technologies, Wilmington, DE) using a Waters Ultrahydrogel Linear 6–13 μm, 7.8 mm × 300 mm column (Waters, Milford, MA). Flow rate was 0.3 mL/min and sample injection volume of 20 μL. Control and integration was done using a refractive index detector and PC with ChemStation (HP ChemStation for LC Rev. A.04.01).

2.5. Starch oxidation

Low pressure size exclusion column (LP-SEC) was used to obtain different fractions (based on molecular weight) of starch components. Sepharose CL-2B was used as the packaging material for the column (1.6 cm × 100 cm). Starch sample (5 mg/mL) was dissolved in 1 M NaOH solution and centrifuged at 134 × *g* for 15 min. Sodium hydroxide (10 mM) solution was used as a buffer. Each fraction was collected in an auto sampler and every third fraction was analyzed further. Each fraction was collected for 9 min. Flow rate was 0.27 mL/min.

Amount of oxidation in the different fractions of starch was determined by the uronic acid assay developed by Blumenkrantz and Asboe-Hansen (1973). Starch fraction (200 μL) was dissolved in 1.2 mL of sulfuric acid and cooled in crushed ice for 5 min. Afterwards, the sample was mixed in a vortex mixer for 5 s and heated in water bath at 100 °C for 5 min. The sample was cooled in an ice water bath for 5 min, and 20 μL of *m*-hydroxydiphenyl reagent was added. Absorbance was measured at 492 nm after mixing the sample for 5 min.

Total carbohydrate content for different fractions was determined by phenol/sulfuric acid assay as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956) method with some modifications as described by Simsek, Ojanen-Reuhs, Stephens, and Reuhs (2007). Fractions were collected (9 min/fraction, 2 mL) and every third fraction was selected for analysis of total carbohydrates content by phenol–sulfuric acid assay. To determine total carbohydrates in the fractions a portion (100 μL) of every third fraction was aliquoted into test tubes and 100 μL of 5% phenol solution and 500 μL of concentrated sulfuric acid were added. The tubes were placed into a boiling water bath for 10 min. After cooling; the absorbance was read at 490 nm. A standard curve was prepared using a stock solution of 1 mg/mL: glucose. Dilutions of the stock solution were made to produce standards with concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. The standard samples were analyzed along with the fractions and the absorbance was read at 490 nm (R^2 values were always >0.98). Dubois method (1956) is sensitive enough to measure the starch concentrations in our samples since this method could be used to quantify carbohydrates in submicro amounts. For uronic acid determination sodium borohydride in sulfuric acid was added to every third fraction (100 μL). After boiling for 5 min the fractions were cooled on ice and 0.15% *m*-hydroxydiphenyl in 0.5% sodium hydroxide was added. The absorbance of the fractions was read at 492 nm after resting for 5 min.

2.6. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

Detection of carboxyl and carbonyl groups and molecular weight properties of starch polymers were analyzed using ^{1}H NMR, VarianUnity Inova 500 MHz ^{1}H NMR spectrometer (Varian Inc., Palo Alto, CA). Samples were dissolved in 1 mL of deuterium oxide (D_2O) and left at room temperature (20–22 °C) for 2 h and lyophilized. Samples were then freeze-dried (−18° C). The samples were dissolved in 0.6 mL D_2O and freeze-dried (−18° C) again and transferred into NMR tubes. ^{1}H NMR spectra were obtained at 80 °C. Starch isolated from non-ozone-treated flour was regarded as control sample.

2.7. Physical properties

Scanning electron microscopy was conducted at the Electron Microscopy Center located at North Dakota State University, Fargo. Samples were mounted on aluminum mounts with silver paint. After attachment to the mounts, the samples were coated with gold–palladium using a Balzars SCD030 sputter coater. The specimens were examined and photographed using a JEOL JSM-6300 scanning electron microscope.

Relative degree of crystallinity of starch samples was investigated by X-ray powder diffraction (Philips vertical Multi-Purpose Diffractometer PW3040) operating at 50 kV and 40 mA (Cu-Kα radiation of 0.154 nm). The degree of crystallinity was investigated as described before (Chakraborty, Matkovic, Grier, Jarabek, Berzonsky, & McMullen, 2004). The starch samples were wrapped in aluminum pans and diffracted intensity was measured from 5° to 35° as a function of 2θ. A scan range of 5–35° 2θ was selected to avoid the aluminum peaks (from aluminum pans) and input from the starch material at high angles yielded little valuable information. The degree of crystallinity of the sample was defined by the intensity ratio of the diffraction peaks and of the sum of all measured intensity using PANalytical software X'Pert HighScore v. 2.2c. The constant background intensity arising from imperfections of the sample, the X-ray optics of the instrument, and sample fluorescence and scatter was subtracted from the total intensity. Degree of crystallinity was calculated using the following equation:

$$\text{Crystallinity } [\%] = 100 \times \frac{\sum I_{\text{net.}}}{\sum I_{\text{tot.}} - \sum I_{\text{cons.bgr.}}} \quad (1)$$

The standard reference material was Respirable Alpha Quartz (NBS 1878, 95.5% crystallinity), which determined the constant background. All backgrounds were determined by using exactly the same automatic setting (granularity = 20, use smoothed input data = yes, bending factor = 6).

The DSC analysis of the starch samples was done using Perkin-Elmer DSC-7 (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA) with slight modifications to the method described by White, Abbas, Pollak, and Johnson (1990). Data obtained was analyzed with thermal analysis data station. Samples (3.5 mg, as is) were weighed into aluminum pans and deionized water (0.8 μL) was added. The aluminum pans were sealed and kept overnight at room temperature (20–22 °C). Aluminum pan containing deionized water (0.8 μL) was regarded as the standard. Each sample was heated under nitrogen gas from 10 to 120 °C at 10 °C/min. Parameters recorded were enthalpy of gelatinization (ΔH), onset (T_o), peak (T_p), and conclusion (T_c) temperatures. All the analyses were done in triplicate.

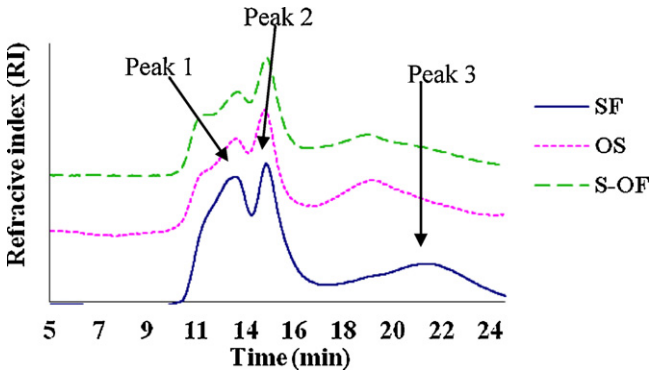

Fig. 2. Molecular weight distribution of polysaccharides from starch isolated from control flour (SF), starch isolated from ozonated flour (S-OF), and starch isolated from control flour followed by ozonation (OS). The high (peak 1) and medium (peak 2) molecular weight amylopectin and amylose (peak 3) are presented in the HPSEC chromatograph.

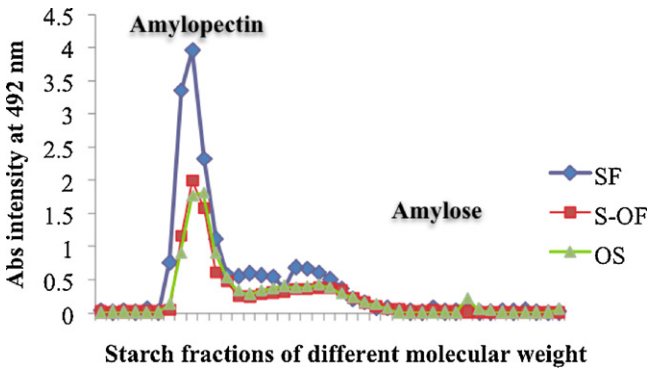

Fig. 3. Effect of ozonation on total carbohydrate content of ozone and no ozone treated starch fractions. Starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS).

2.8. Swelling power and pasting properties

Swelling power was performed on starch samples following the method described by McCormick, Panozzo, and Hong (1991). Starch (0.2 g, as is) was weighed into heat stable plastic culture tubes (VWR North American Cat. No 89039-668) (95 mm × 17 mm). Distilled water (5 mL) was added to the samples and then mixed (Vortex mixer) for 10 s and placed in a shaking water bath at 70 °C for 4 min, mixed (Vortex mixer) again for 20 s and placed in the water bath for 6 min. The samples were then transferred to a boiling water bath for 10 min, then placed in ice water for 5 min, and lastly centrifuged at 1,643 × *g* for 4 min. The supernatant was decanted and the sediment was weighed. Swelling power was determined as sediment weight divided by dry sample weight.

The pasting properties of starch samples were determined using a Rapid Visco Analyzer (RVA) model 4SA (Newport Scientific; Warriewood NSW, Australia) with slight modifications to the method described by Chakraborty et al. (2004). The equipment was interfaced with computer equipped with Thermocline and Thermoview software (Newport Scientific, Warriewood, NSW, Australia). Starch (3 g, based on 14% m.b.) was added to deionized distilled water (25 mL, based on 14% m.b.) in an RVA canister. The rate of heating and cooling was 12 °C/min, idle temperature was 50 °C. Total run time for each sample was 13 min. Parameters recorded were peak viscosity (PV), hot paste viscosity (HPV), breakdown (BKD), cold paste (CPV) and setback (STB) viscosity. All measurements were reported in Rapid Visco Units (RVU).

2.9. Experimental plan

Experimental design was a randomized complete block. The three commercial flours were treated as three replicates. Experimental units (3) consisted of starch isolated from control flour (S-F); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS). Data were subjected to an analysis of variance. *F*-tests were significant at $P < 0.05$. Means were separated using Fisher's protected LSD at $P = 0.05$.

3. Results and discussion

3.1. Amylose/amylopectin content

Typical HPSEC chromatograms are shown in Fig. 2. Molecular weight of eluted molecules decreases from left to right. Hence, the prominent peaks in the center of the chromatogram represent amylopectin (peak 1 and peak 2) and the smaller peak on the right represents amylose (peak 3). The peaks were identified based on work done by Grant et al. (2002). The first peak was more pronounced for starch exposed to ozone either directly (OS) or starch from ozonated flour (S-OF) than for control starch (SF). The areas under the amylopectin peaks (peak 1) were greater for non-ozone-treated starch (SF: 40.1%) than for ozone treated samples (S-OF: 35.4% and OS: 36.0%) (data not shown). These results indicated hydrolysis of amylopectin content resulting in depolymerization of amylopectin fractions. Conversely, the amylose peak (peak 3) was greater for ozone treated samples (S-OF: 33.0% and OS: 34.4%) than for control (SF: 28.9%). The amylopectin eluted at the same time for all samples and was represented by two peaks, i.e. high molecular weight amylopectins (peak 1), and medium molecular weight amylopectins (peak 2). Furthermore, the amylose peak eluted earlier for OS and S-OF (~19 min) as compared to SF (~21.6 min), which indicates an increase in area under high molecular weight amylose. The reduction in area under amylopectin and increase in area under amylose content is attributed to hydrolysis of amylopectin chains by ozone. Hydrolyzed amylopectin branch chains would behave like amylose and thus would result in an increase in apparent amylose content as observed for S-OF and OS (Fig. 2). Depolymerization of amylopectin is in agreement with the findings of Kesselmans, Veer, Brouwer, and Wielema (2004), who reported that ozone oxidation of corn starch fractions caused partial depolymerization of starch polymers.

3.2. Starch oxidation

The observed reduction in amylopectin and increase in lower molecular weight polydispersed starch polymers is supported by the total carbohydrate content found in fractions collected by LP-SEC. Results showed a decrease in total carbohydrate content in amylopectin fractions of S-OF and OS as compared to those of SF. Phenol–sulfuric acid assay was used to determine the total carbohydrate content in starch fraction (Fig. 3). Thus, the lower carbohydrate content is attributed to oxidation of sugars and/or to partial depolymerization of amylopectin chains (Kesselmans et al., 2004; Murphy, 2000).

Uronic acid assay test was used to determine the amount of carboxylic acids produced in these fractions as a result of oxidation of starch. The S-OF and OS had higher amount of carboxylic groups in the amylopectin fractions than did the SF (Fig. 4). These results were in accordance with Chan et al. (2009), who reported the formation of carboxyl (COOH) and carbonyl (C=O) groups in ozone oxidized corn, sago and tapioca starches. Wurzburg (1986) also reported an increase in carboxyl content of commercial starches oxidized with hypochlorite. The hydroxyl groups present at C2, C3, and C6

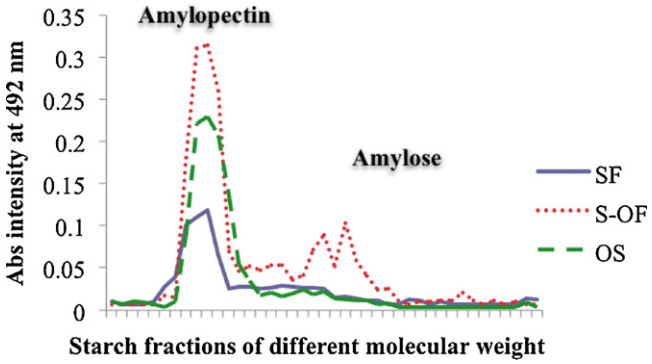

Fig. 4. Effect of ozonation on carboxylic acid content of ozone and no ozone starch fractions. Starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS).

positions on glucose molecule have been reported to be susceptible to oxidation with the formation of bulky carboxyl and carbonyl groups and partial depolymerization of starch granules (Kesselmans et al., 2004; Murphy, 2000).

Oxidation of hydroxyl groups was confirmed by ^{1}H NMR analysis (Figs. 5 and 6). The resonance peaks derived from ^{1}H NMR studies were identified based on work done by Salomonsson, Anderson, Torneport, and Theander (1991) on bromine oxidized starch. The resonance at 3.85 ppm (approx.) for S-OF, as compared to SF sample, indicates the presence of carboxylic acid (Fig. 5). Oxidized starch produced several resonances between 3.5 and 4.0 ppm, as compared to SF (control) sample, indicating depolymerization and formation of carboxylic acid (Fig. 5). Similarly resonances appearing in S-OF and OS samples at 3.25 ppm (approx.) arise from hydrolysis at H2 terminal (proton at C-2 position) and the formation of β-glucuronic acid (Fig. 5). Magnification of signals between 5.6 and 4.6 ppm showed more differences between ozone treated and non-treated starch samples (Fig. 6). No significant resonances were observed between 5.4 and 5.8 ppm in SF (control) sample; however, significant resonances (5.48–5.70 ppm) were observed in S-OF and OS samples. Appearances of these resonances correspond to the formation of carboxylic acid. Resonance at 5.70 ppm corresponds to the formation of a keto group [(1→4)-3-keto] at the H1 terminal (proton at C-1 position) and the resonance at 5.48 ppm indicates the formation of a (1→4) glucuronic acid at H1 terminal (proton at C-1 position), which comes from carboxylic acid formation.

No resonances were observed at 5.26 ppm in SF (control) sample; however, due to carboxylic acid (α-glucuronic acid) formation at H1 terminal (proton at C-1 position), a significant increase in resonance intensity was observed at 5.25 ppm in S-OF and OS samples. Formation of α-glucuronic acid also indicates that depolymerization of starch occurred, which supports the results of SE-HPLC analysis that showed a decline in area under amylopectin peak (peak 1) and an increase in area under amylose peak (peak 3) (Fig. 2).

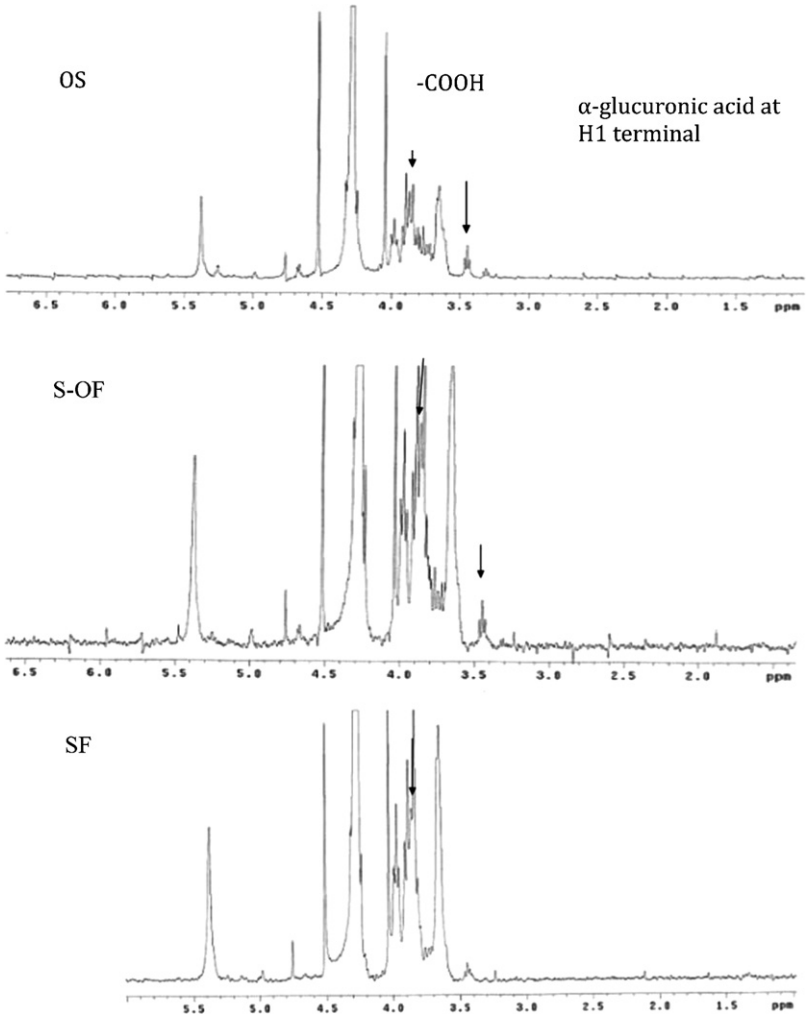

Fig. 5. ^{1}H NMR spectrum (1.5–6.5 ppm) of starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS).

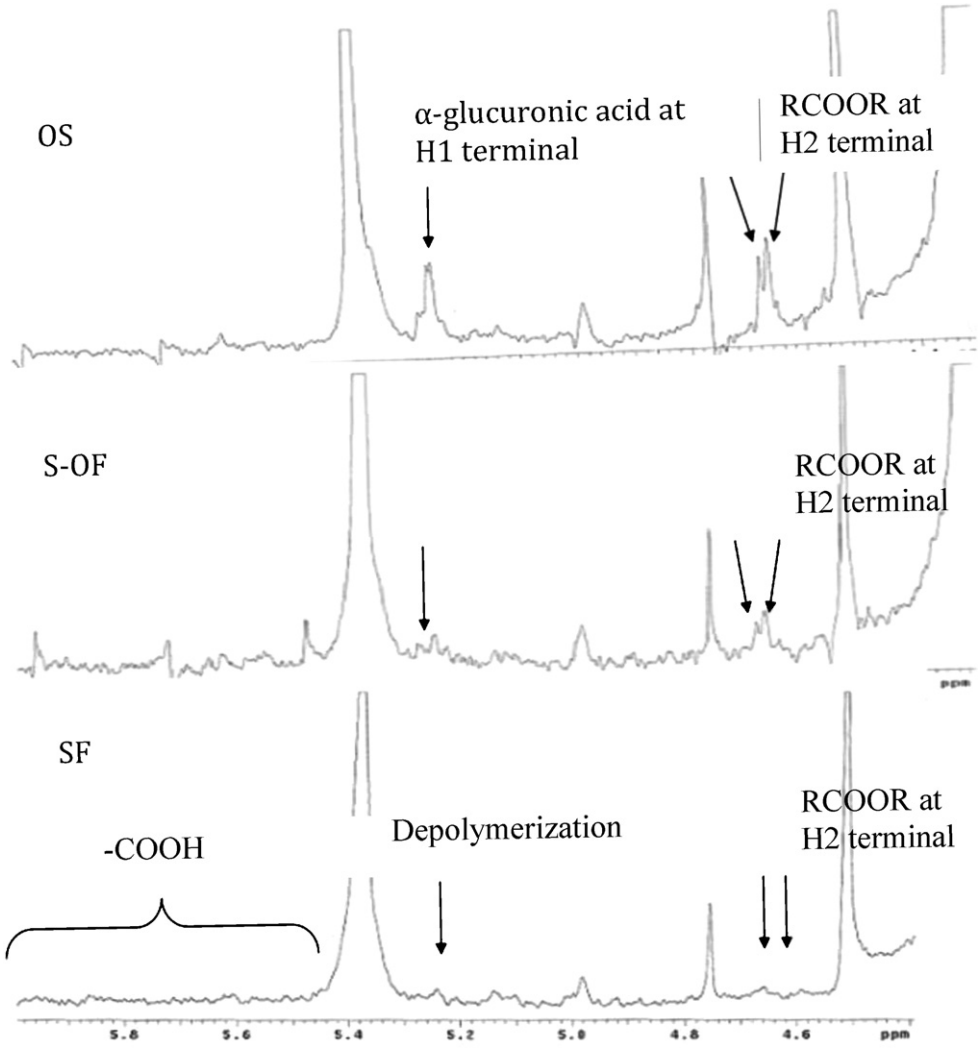

Fig. 6. ^{1}H NMR spectrum (4.6–5.8 ppm) of starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS).

The resonance appearing in S-OF and OS samples at 4.66 ppm and 4.64 ppm indicate the formation of keto group [(1→4)-3 keto] at H-2 terminal (proton at C-2 position) and β-glucuronic acid at H-1 terminal (proton at C-1 position), respectively. These results clearly indicate that ozone treatment caused oxidation of isolated starch and starch from treated flour, which resulted in the formation of carboxyl and keto groups and ultimately in partial depolymerization of starch. Previous research has also reported the formation of carboxyl and carbonyl groups in ozone treated corn, sago and tapioca starch (Chan et al., 2009).

3.3. Physical properties

Scanning electron micrographs did not reveal any visible differences in granule morphology between ozone treated and non-ozone treated samples. The starch granules were intact with no apparent damage to the surface of granule (micrographs not shown).

X-rays can penetrate to the aluminum pan substrate, producing undesired peaks at higher angles. Also, the contribution from starch material at high angles yielded little valuable information. The peaks in the range between 5° and 35° 2θ were used to calculate the relative degree of crystallinity. The crystal structures were derived from studies on amylose (Imberty, Buleon, Tran, & Perez, 1991). The amylopectin crystallizes within the granule and its side chain branches interwine to form the double helices that are the basis of the crystals. X-ray diffraction showed the typical A-type diffraction pattern for cereal starches (Paris, Bizot, Emery, Buzare, & Buleon, 1999). No significant differences were found in crystallinity of ozone treated and non-ozone treated samples (Fig. 7). The calculated degree of crystallinity for SF (control), S-OF and OS samples were 8.24%, 8.84%, and 8.41%, respectively (data not shown).

Thermal properties were not affected by ozone treatment. Ozone treatment did not affect transition temperatures of starch samples as measured by DSC (data not presented). Similarly, gelatinization change (ΔH) were not significantly different among S-OF (8.8 J/g), OS (9.5 J/g) and SF (7.2 J/g) starch isolates (data not shown). These results are in agreement with Chittrakorn (2008), who reported that ozone treatment did not affect the transition temperatures or enthalpies (ΔH) of soft wheat flour samples as measured by DSC. Allen, Sherbon, Lewis, and Hood (1982) also reported that oxidation using a chlorine treatment did not affect the transition temperatures and enthalpies of wheat flour and starch samples as measured by DSC.

The enthalpy change (ΔH) is the energy required to dissociate or disrupt the ordered structure of granules during gelatinization.

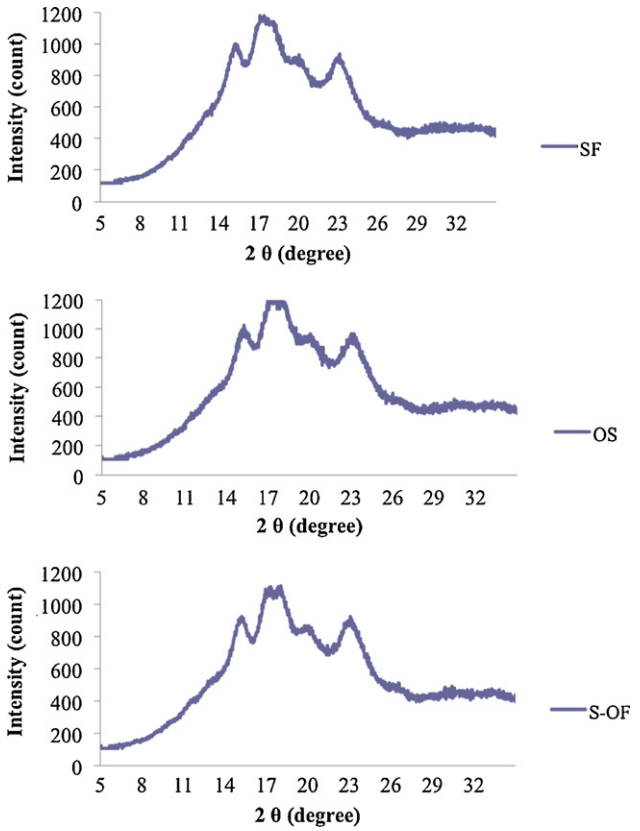

Fig. 7. X-ray diffraction patterns of starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS).

The breakdown of crystalline order and molecular order (double helices) during gelatinization results in change in ΔH (Cooke & Gidley, 1992). The lack of effect on ΔH agrees with the X-ray diffraction data that indicated that ozone had no effect on the crystallinity of the starch granules.

3.4. Pasting properties

Results from the swelling power test (Fig. 8) confirm that OS (8.5%) and S-OF (8.4%) had greater swelling power than did control starch (7.5%). Chan et al. (2009) reported a positive correlation ($r = 0.63$, $P < 0.01$) between carboxyl content and swelling power of ozone oxidized corn starch. When starch is heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. The increase in swelling power of ozone treated starch granules during heating in water might be due to hydrophilic carboxyl groups (–COOH) and the repulsion between negative charges. Strong interchain hydrogen bonding between the

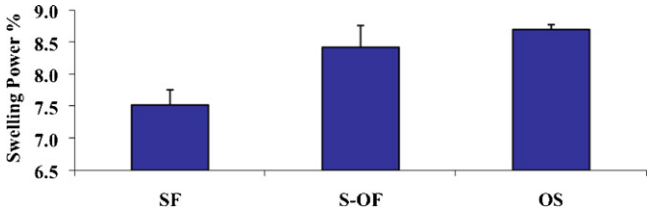

Fig. 8. Effect of ozonation on swelling power of isolated starch. Starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); starch that was ozonated after isolation from control flour (OS).

Table 1
Effect of ozonation on pasting profiles of ozone and non-ozone treated isolated starch.

Sample[a]	Peak viscosity	Breakdown	Final viscosity	Setback
SF	198 a	38 a	245 a	84 a
S-OF	216 a	49 b	272 a	104 a
OS	205 a	50 b	241 a	86 a
LSD	27	9	39	30

Starch isolated from control flour (SF); starch isolated from ozonated flour (S-OF); and starch that was ozonated after isolation from control flour (OS). Values are means of three replicates. LSD = least square difference. Means followed by different letters in the same column are significantly different ($P < 0.05$).

carboxyl groups (–COOH) and the hydroxyl groups of the amylose and amylopectin results in formation of strong network, which can hold more water (Athawale & Lele, 2001). LP-SEC and ^{1}H NMR studies indicated an increase in carboxyl and carbonyl content of ozone treated wheat starch (Figs. 4–6).

The pasting properties of ozonated and non-ozonated starch samples are given in Table 1. Peak viscosities for S-OF, OS, and SF samples were not significantly different. This is in agreement with the lack of affect on thermal properties as determine by DSC. Final viscosity and setback viscosities were not significantly different for S-OF, OS, and SF (Table 1). Peak time of SF, S-OF and OS were not significantly different (data not presented). Breakdown values of S-OF and OS increased significantly as compared to SF samples, indicating that starch granules exposed to ozone were more susceptible to breakage during heating (Table 1). Breakdown values reflect the loss of viscosity or stability of starch paste during heating and stirring the hot paste at 95 °C. The loss of viscosity occurs as starch polymers align with shear field. The results were in accordance with the findings of An and King (2009) who reported an increase in breakdown of viscosity of ozone oxidized rice starch. Chan et al. (2009) reported a negative correlation ($r = -0.86$, $P < 0.01$) between carboxyl content and breakdown viscosity and concluded that the decline in viscosity might reflect the weakening of starch granules due to the degradation of amorphous regions of the oxidized starch granules. LP-SEC and ^{1}H NMR studies showed an increase in carboxyl content of ozone treated wheat starch (Figs. 4–6), which might have resulted in decreased breakdown viscosity.

4. Conclusion

Starch contributes to the textural properties of many foods and is used in food industry as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent. Limitations such as low shear resistance, thermal resistance, thermal decomposition and high tendency towards retrogradation limit the use of native starch in some industrial food applications. Starch modification, alters the physical and chemical characteristics of the native starch and improves its functional characteristics and usage in food industry. Modified starches such as oxidized starches are used in food products where low viscosity, high clarity, and low temperature stability is required in foods. Oxidized starches are used in batters and breading for coating of various foods, in confectionery as binders and film formers, in dairy as texturizers, in salad dressing and in mayonnaise. Different types of chemicals have been used to oxidize starches whereas ozone could be used as a “green oxidant” to oxidize starch as ozone does not leave any residue when it is introduced to a food product.

Results indicate that isolated starch and starch in flour were oxidized by ozone gas. Oxidation from ozone treatment of flour and isolated starch caused partial depolymerization of high molecular weight amylopectins and thus producing low molecular weight starch polymers and amylose. Oxidation of starch also resulted in higher amount of carboxylic groups in amylopectin fractions of

ozone treated starch isolates as determined by uronic acid test and by ^{1}H NMR analysis. ^{1}H NMR results indicated formation of keto group [(1→4)-3 keto] at H-2 terminal (proton at C-2 position) and β glucuronic acid at H-1 terminal (proton at C-1 position). The negative charges of carboxyl groups repel each other causing increased swelling of starch granules during heating in water and subsequent increase in paste breakdown. Visual analysis via scanning electron microscopy indicated no effects from ozone treatment. Similarly, X-ray diffraction and change in gelatinization from DSC both indicate that starch granule crystallinity was not affected by ozone treatment.

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