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Physicochemical and Functional Properties of Ozone-Oxidized Starch

Hui T. Chan, Rajeev Bhat, and Alias A. Karim*

Food Biopolymer Research Group, Food Technology Division, School of Industrial Technology,
Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

The effects of oxidation by ozone gas on some physicochemical and functional properties of starch (corn, sago, and tapioca) were investigated. Starch in dry powder form was exposed to ozone for 10 min at different ozone generation times (OGTs). Carboxyl and carbonyl contents increased markedly in all starches with increasing OGTs. Oxidation significantly decreased the swelling power of oxidized sago and tapioca starches but increased that of oxidized corn starch. The solubility of tapioca starch decreased and sago starch increased after oxidation. However, there was an insignificant changed in the solubility of oxidized corn starch. Intrinsic viscosity [η] of all oxidized starches decreased significantly, except for tapioca starch oxidized at 5 min OGT. Pasting proper- ties of the oxidized starches followed different trends as OGTs increased. These results show that under similar conditions of ozone treatment, the extent of starch oxidation varies among different types of starch.

INTRODUCTION

Many industries use oxidized starch to provide surface sizing and coating properties (e.g., the paper, textile, laundry finishing, and building materials industries) (*1*). Oxidized starch has several unique functional properties, such as low viscosity, high stability, clarity, film forming, and binding properties (*2*). It has been applied to foods as a coating and sealing agent in confectionaries, an emulsifier (*3*), a gum arabic replacer (*4*), a dough conditioner for bread (*5*), and a binding agent in batter. These types of starches provide good adhesion of the batter to food and a crispy texture after frying (*6*).

Oxidized starch is produced by reacting starch with a specified amount of oxidizing reagent under controlled temperature and pH (*7*). Hypochlorite oxidation is the most frequently used method for oxidation of starch, while sodium bromide (NaBr), gaseous chlorine, calcium hypochlorite, hydroperoxide, potas- sium permanganate, ammonium persulfate (*8*), oxygen, and bromide (*9*) are used less frequently. Following oxidation, the relatively bulky carboxyl (COOH) and carbonyl (C=O) groups are introduced together with partial depolymerization of the starch chains (*10*). According to Wurzburg (*7*), the bulky carboxyl group on the starch molecules results in low retrogradation of oxidized starch paste, whereas the carbonyls play a minor role in the prevention of retrogradation of starches. Hydrolysis of glycosidic bonds causes a loss of polymer properties and de- creased viscosity (*11*).

Chemical oxidizing agents generally are objectionable for starch modification because of safety and environmental issues. For example, large amounts of salts are formed in the

hypochlorite oxidation process, which causes wastewater disposal problems (*12*). In contrast, ozone does not leave a residue when it is introduced to a food product. Therefore, ozone treatment would be a good alternative to chemical treatment of food products. Ozone is a naturally occurring and industrially gener- ated form of oxygen (*13*). It is a more powerful oxidant than oxygen due to its extra oxygen atom, which it can share with other substances to oxidize them. Moreover, it is a clean oxidant that has an elevated thermodynamic oxidation potential, which means that the reactions can be performed at low temperatures (*14*).

Several patents have been filed for a method of oxidizing dry starch (*12*) and polysaccharides (*15*) using ozone as an oxidizing agent, but a scientific report about the effect of ozone treatment on starches is lacking. Therefore, the aim of the current study was to characterize the effect of ozone treatment on the physicochem- ical and functional properties of corn, sago, and tapioca starches.

MATERIALS AND METHODS

Materials. Corn, tapioca, and sago starch were obtained from Sim Company Sdn. Bhd. (Penang, Malaysia). All other reagents used in this work were of analytical grade.

Preparation of Ozone-Oxidized Starches. Ozone (O_3) oxidation was carried out in a rotating vessel designed for an evaporator that was connected to an ozone generator (SA-100P Ozonizer, Ishimori Seisakusho Co. Ltd., Japan) and an oxygen (O_2) cylinder (**Figure 1**). Ozone was generated by corona discharge reactor cells in which diatomic oxygen was flowed though a high-voltage electric field produced between conductive and dielectric surfaces. The reaction vessel, generator, and O_2 cylinder were connected by Teflon tubing, and all connections were firmly sealed. The flow rate of O_2 gas from the cylinder was 8 ± 0.2 mL/s, which was determined by a bubble type flow meter. This flow rate was assumed to be the rate of O_3 flow to the reaction vessel. Two potassium iodide (KI) traps were placed at the end of the reaction vessel to trap unreacted ozone.

*To whom correspondence should be addressed. Tel: +604 653 2268. Fax: +604 657 3678. E-mail: akarim@usm.my.
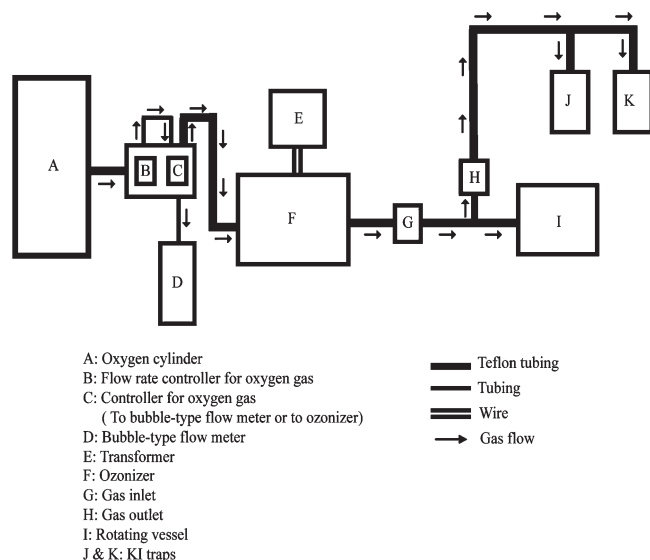
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A: Oxygen cylinder
B: Flow rate controller for oxygen gas
C: Controller for oxygen gas
  (To bubble-type flow meter or to ozonizer)
D: Bubble-type flow meter
E: Transformer
F: Ozonizer
G: Gas inlet
H: Gas outlet
I: Rotating vessel
J & K: KI traps

━━━ Teflon tubing
──── Tubing
═══ Wire
──▶ Gas flow

Figure 1. Schematic diagram for the setup of the ozone oxidation system.

The concentration of the KI solution was 4%, and an appropriate amount (total 1 L) was placed in each trap to react with O_3 by following equation:

$$O_3 + 2KI + H_2O \rightarrow O_2 + I_2 + 2KOH \qquad (1)$$
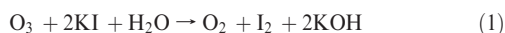
To perform the reaction, a starch sample (in the powder form, "as is" moisture content) was placed in the reaction vessel. O_3 was generated for 1, 3, 5, and 10 min ozone generation times (OGTs), and the reaction vessel was rotated at 150 rpm to ensure homogeneous contact between starch and O_3 during the reaction. Next, 10 min of contact time elapsed to allow the reaction (oxidation) to take place between O_3 and starch with both the gas inlet and the outlet closed. Finally, O_2 was flushed through the vessel for 20 min to flush out O_3 that did not react with the starch. When this procedure was completed, the oxidized starch was removed from the vessel. The amount of O_3 that did not react with the starch was determined by titration of the KI solutions in the traps. Approximately 10 mL of 2 M H_2SO_4 was added to the KI solution, and the amount of I_2 released via the reaction shown in eq 1 was determined by titration with a standardized 0.2 M $Na_2S_2O_3$ solution (by using starch solution as the indicator) as follows:

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \qquad (2)$$

Therefore, 1 mol of $Na_2S_2O_3$ consumed was equivalent to 0.5 mol of ozone. Prior to the reaction of starch with O_3, a blank test was conducted following exactly the same procedure but without starch. A calibration curve was obtained by plotting the amount of O_3 generated (A) against OGT (t). The amount of O_3 reacted with starch was calculated by subtracting the unreacted amount from the amount generated. Duplicate samples for each OGT were prepared from each starch type.

Carbonyl Content (%). The carbonyl group content was determined following the titrimetric method of Smith (*16*). The carbonyl group content was calculated as follows:

$$\text{percentage of carbonyl content} = [(\text{blank} - \text{sample}) \text{ mL}$$
$$\times \text{ acid normality} \times 0.028$$
$$\times 100]/\text{sample weight (dry basis) in g}$$
$$(3)$$

Carboxyl Content (%). The carboxyl content of oxidized starch was determined according to the modified procedure of Chattopadhyay et al. (*4*). A starch sample (2 g) was mixed with 25 mL of 0.1 mol equiv/L HCl, and the slurry was stirred constantly for 30 min with a magnetic stirrer. The slurry then was vacuum filtered through a 150 mL medium porosity fritted glass funnel and washed with 400 mL of distilled water. The starch cake was carefully transferred into a 500 mL beaker, and the volume was adjusted to 300 mL with distilled water. The starch slurry was heated in a boiling water bath with continuous stirring for 15 min to ensure complete

gelatinization. The hot starch dispersion was then adjusted to 450 mL with distilled water and titrated to pH 8.3 with standardized 0.01 mol equiv/L NaOH. A blank test was performed with unmodified starch. The carboxyl content was calculated as follows:

$$\text{mequiv of acidity}/100 \text{ gofstarch} = [(\text{sample} - \text{blank}) \text{ mL}$$
$$\times \text{ normality of NaOH}$$
$$\times 100]/\text{sample weight (dry basis) in g}$$
$$(4)$$

$$\text{percentage of carboxyl content} = [\text{mequiv of acidity}/100 \text{ gofstarch}]$$
$$\times 0.045$$
$$(5)$$

Swelling Power and Solubility. Swelling power and solubility of starch samples were determined according to the method of Schoch (*17*). Swelling power is the ratio of the weight of the wet sediment to the initial weight of dry starch. The solubility is the ratio in weight of the dried supernatant to the initial weight of dry starch.

Intrinsic Viscosity. Intrinsic viscosity was determined based on the method developed by Ahmad et al. (*18*) with minor modification. A 0.5% (w/w) starch suspension was prepared by dissolving starch in 90% dimethyl sulfoxide (DMSO). The sample was incubated in an incubator shaker (150 rpm) at room temperature for 16 h. The intrinsic viscosity measurement of all samples was conducted using an Ubbelohde type capillary viscometer (Poulten Self-e & Lee Ltd., Essex, United Kingdom; PSL ASTM- IP IC, constant = 0.03009 $[\text{mm}^2/\text{s}]/\text{s}$) with a 0.75 mm diameter in a constant temperature water bath at 25.0 ± 0.1 °C. Exactly 10 mL of starch solution was transferred to the viscometer and held for 5 min to equilibrate temperature. The mean of three readings was taken. The samples were further diluted with 90% DMSO to yield four different concentrations within the range of 0.3−0.5%, w/w. The intrinsic viscosity of the starch solution at an infinite dilution was obtained by extrapolating the specific viscosity values measured for the successive dilutions.

Pasting Properties of Starch. The pasting profile of the starches (8% w/w, dry weight basis) was determined using the Rapid Visco Analyzer (model RVA-4, Newport Scientific Pvt. Ltd., Warriewood, Australia). The samples were equilibrated at 50 °C for 1 min and then raised to 95 °C in 3.75 min, held for 2.5 min, cooled to 50 °C in 3.75 min, and held for 5 min. The paddle speed was set at 960 rpm for the first 10 s to evenly disperse the starch slurry and reduced to 160 rpm throughout the remainder of the experiment. The units of viscosity were expressed as rapid visco units (RVUs).

Statistical Analysis. Duncan's least significant test was used to compare means at the 5% significance level. Simple Pearson's correlation and regression analysis was conducted using SPSS 15.0 software (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

In the following discussion, the term "unmodified starch" refers to the starch that has not undergone any form of modification. Oxidized starch (corn, sago, or tapioca) refers to starch samples that were oxidized with ozone gas for 1, 3, 5, or 10 min OGTs.

Ozone Oxidation. Table 1 shows that in general, the amount of ozone that reacted with the starches increased as OGT increased. A longer OGT meant that more ozone gas was being produced and trapped in the reaction vessel. The results suggest that as the OGT increased, the amount of ozone that reacted with starch also increased due to a higher oxidation level of the starch.

Carboxyl and Carbonyl Content. Table 2 shows the carboxyl and carbonyl contents of oxidized corn, sago, and tapioca starches at different OGTs. The content of both compounds increased in oxidized starches as OGT increased. The carboxyl content of oxidized starches treated under the experimental conditions ranged from 0.001 to 0.063%. This value is lower than those reported by Wurzburg (*7*), who found that the carboxyl content of most commercial starches oxidized with

hypochlorite can reach about 1.1%. According to Murphy (*10*), during oxidation, the relatively bulky carboxyl (COOH) and carbonyl (C=O) groups are introduced together with partial depolymerization of the starch chains. Pearson correlation of our data shows a significant positive relationship between the amounts of ozone reacted with starches and carboxyl (corn, $r =$ 0.924; sago, $r = 0.768$; and tapioca, $r = 0.910$, $P < 0.01$) and carbonyl contents (corn, $r = 0.914$; sago, $r = 0.627$; and tapioca, $r = 0.887$, $P < 0.01$). Our results agree with the finding by Kuakpetoon and Wang (*19*), who reported that both carboxyl and carbonyl contents of oxidized starch increased as the hypochlorite concentration increased.

Both carboxyl and carbonyl contents varied significantly according to starch type (**Table 2**). There was a significant difference in carbonyl content between corn, sago, and tapioca starches at all levels of oxidation. Tapioca starch had the highest carbonyl content, whereas corn starch had the lowest at all oxidation levels. On the other hand, corn starch had the highest carboxyl content at 1 and 10 min OGT and the lowest at 3 and 5 min OGT as compared to other starches. These differences could be attributable to the different structural and morphological characteristics of the starches studied. The higher carboxyl content of corn probably is associated with the presence of natural pores on corn starch granules; the pores seem to allow more ozone gas to diffuse into the starch granules. The inconsistent trend of carboxyl content of ozone-oxidized starch at different OGTs could be due to different rates of depolymerization of starch polymer chain to produce carbonyl group and the rate of oxidation of carbonyl group to carboxyl group. The bulky carboxyl group on the starch molecules can result in low retrogradation of oxidized starch paste, whereas the carbonyls play a minor role in the prevention of retrogradation of starches (*7*). The carbonyl contents of all oxidized starches were higher than the carboxyl contents at the same oxidation level, except for corn

at 10 min OGT. Most of the literature reports that chemically oxidized starches have a higher carboxyl content than carbonyl content. According to Wurzburg (*7*), aldehydic reducing end groups on the amylose and amylopectin generally are more readily oxidized to carboxyl groups as compared to hydroxyls groups. In our case, it is possible that the ozonation conditions used were not sufficient to completely oxidize the aldehydic reducing groups.

Swelling Power and Solubility. Swelling power and solubility are measures of the magnitude of the interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose-to-amylopectin ratio and phosphorus content and by the characteristics of the amylose and amylopectin in terms of molecular weight/distribution, degree of branching and branch length, and conformation (*20*, *21*). **Tables 3** and **4** present the swelling power and solubility data for the unmodified and oxidized starches, respectively. From 1 to 5 min OGT, the swelling power for oxidized corn starches was inconsistent but increased significantly at 10 min OGT. As mentioned in the preceding discussion, the inconsistency could be attributed to the different rates of depolymerization of the polymer chain to form a carbonyl group and oxidation of carbonyl group to carboxyl group at different OGTs. For tapioca and sago starches, the rate of depolymerization appears to be greater than oxidation of the carbonyl group to the carboxyl group. The increase in swelling power of oxidized starch might be due to the introduction of hydrophilic carboxyl groups and the repulsion between negative charges. This is consistent with results of the correlation coefficient analysis, which shows a significant positive correlation between the carboxyl content and the swelling power of oxidized corn starch ($r = 0.632$, $P < 0.01$). Furthermore, part of the amylose had presumably been degraded, thereby allowing the starch granule to absorb water and swell more easily when subjected to heat.

In contrast, the swelling power of both oxidized sago and tapioca starch significantly decreased as compared to the unmodified form. The reduction in swelling power after oxidation may be due to structural disintegration within the starch granule during the process of modification (*22*). A similar decrease in the swelling power upon oxidation was reported for the mucuna bean (*23*). Solubility represents the amount of solubilized starch molecules present at a certain temperature. As shown in **Table 4**, the solubility of the unmodified corn starch and all oxidized corn starches did not differ significantly. In contrast, the solubility of oxidized sago starch increased significantly upon oxidation, but solubility did not differ significantly among the oxidation levels (OGTs). According to Hodge and Osman (*24*), the increase

Table 1. Amount of Ozone Reacted with Corn, Sago, and Tapioca Starches (mmol)[a]

OGT (min)	starch		
	corn	sago	tapioca
1	0.47 ± 0.03 d	0.39 ± 0.04 c	0.41 ± 0.03 c
3	0.62 ± 0.02 c	0.67 ± 0.11 b	0.58 ± 0.02 b
5	0.71 ± 0.04 b	0.76 ± 0.05 b	0.66 ± 0.08 b
10	1.30 ± 0.04 a	1.37 ± 0.24 a	0.99 ± 0.09 a

[a] Results are expressed as means ± standard deviations ($n = 3$). Values in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

Table 2. Carbonyl and Carboxyl Contents (%) of Ozone-Oxidized Starches[a]

starch	OGT (min)			
	1	3	5	10
	carbonyl			
corn	0.025 ± 0.005 Cc	0.037 ± 0.005 Cb	0.039 ± 0.006 Cb	0.061 ± 0.006 Ca
sago	0.066 ± 0.010 Bb	0.068 ± 0.011 Bb	0.077 ± 0.008 Bab	0.085 ± 0.011 Ba
tapioca	0.121 ± 0.015 Ab	0.126 ± 0.019 Ab	0.140 ± 0.014 Ab	0.250 ± 0.022 Aa
	carboxyl			
corn	0.011 ± 0.007 Abc	0.006 ± 0.003 Bc	0.020 ± 0.006 Bb	0.063 ± 0.008 Aa
sago	0.002 ± 0.002 Bc	0.008 ± 0.004 ABc	0.037 ± 0.010 Ab	0.048 ± 0.005 Ba
tapioca	0.001 ± 0.001 Bd	0.013 ± 0.002 Ac	0.033 ± 0.009 Abb	0.058 ± 0.005 ABa

[a] Results are expressed as means ± standard deviations ($n = 4$). Values in the same row with the same lowercase letters are not significantly different ($P > 0.05$). Values in the same column separately for carbonyl and carboxyl contents with the same uppercase letters are not significantly different ($P > 0.05$).

Table 3. Swelling Power (g/g) of Ozone-Oxidized Starches[a]

OGT (min)	starch		
	corn	sago	tapioca
unmodified	9.45 ± 0.05 c	9.98 ± 0.19 a	15.27 ± 0.35 a
1	9.68 ± 0.06 ab	9.27 ± 0.52 b	12.49 ± 0.33 b
3	9.52 ± 0.16 bc	9.21 ± 0.14 b	12.03 ± 0.29 b
5	9.59 ± 0.09 bc	9.03 ± 0.45 bc	9.23 ± 0.31 d
10	9.78 ± 0.11 a	8.46 ± 0.12 c	10.51 ± 0.19 c

[a] Results are expressed as means ± standard deviations (*n* = 4). Values in the same column with the same lowercase letters are not significantly different (*P* > 0.05).

Table 4. Solubility (%) of Ozone-Oxidized Starches[a]

OGT (min)	starch		
	corn	sago	tapioca
unmodified	2.81 ± 0.63 a	0.78 ± 0.00 b	4.382 ± 0.00 a
1	2.74 ± 0.30 a	1.33 ± 0.08 a	3.089 ± 0.47 b
3	2.90 ± 0.50 a	1.26 ± 0.09 a	3.330 ± 0.36 b
5	3.19 ± 0.45 a	1.30 ± 0.40 a	3.068 ± 0.51 b
10	3.24 ± 0.34 a	1.30 ± 0.31 a	3.878 ± 1.27 ab

[a] Results are expressed as means ± standard deviations (*n* = 4). Values in the same column with the same lowercase letters are not significantly different (*P* > 0.05).

in solubility after oxidation results from depolymerization and structural weakening of the starch granule. Parovuori et al. (*25*) found that solubility of oxidized potato starch increased as the extent of oxidation progressed. At 1 and 5 min OGT, the solubility of oxidized tapioca starch was significantly reduced. According to Wang and Wang (*26*), a decrease in solubility following oxidation results from the presence of cross-links that prevent the amylopectin molecules from leaching out. Oxidized tapioca starches showed the highest carbonyl content at all OGTs. Thus, it is more possible for cross-linking to occur in tapioca starch than corn or sago starches.

Intrinsic Viscosity. Intrinsic viscosity [η] is essentially a measure of the internal friction or resistance of high polymeric molecules in solution. Higher concentrations of polymeric molecules in solution mean that the internal friction in the solution is greater, which leads to higher [η]. **Table 5** lists the [η] for unmodified and oxidized corn, sago, and tapioca starches at 25.0 ± 0.01 °C. The [η] of all ozone-oxidized starches was significantly lower than that of the unmodified starch as OGT increased. Our results are consistent with those of Martinez-Bustos et al. (*27*), who reported that [η] of starches decreased after oxidation. Noor Fadzlina et al. (*28*) attributed the decrease in [η] values to depolymerization of starch polymers during the oxidation process, which consequently reduced the hydrodynamic volume of the molecules. Tapioca starch at 3 and 10 min OGT had significantly lower [η] than the other starches. At 5 min OGT, tapioca starch had a higher [η] value as compared to that at 3 min OGT. Ozone oxidation reduced the [η] of sago starch, but the difference between the treatments was insignificant. Corn starch treated with the highest oxidation level had the lowest [η] value, but the difference among the 1, 3, and 5 min OGT samples was insignificant. Pearson correlation analysis of oxidized corn starch data showed a significant negative correlation between the amount of ozone reacted with corn starch and intrinsic viscosity (*r* = −0.896, *P* < 0.01).

Pasting Properties. The pasting properties of unmodified and oxidized corn, sago, and tapioca starches analyzed with a Rapid Visco Analyzer are shown in **Table 6**. The pasting temperature of oxidized corn and sago starches decreased slightly but signifi-

Table 5. Intrinsic Viscosity of Ozone-Oxidized Starches[a]

OGT (min)	starch		
	corn	sago	tapioca
unmodified	218.62 ± 1.11 a	109.51 ± 15.68 a	204.550 ± 4.09 a
1	113.87 ± 12.51 b	57.10 ± 10.66 b	56.493 ± 18.73 c
3	111.27 ± 6.50 b	61.17 ± 16.36 b	33.528 ± 11.45 d
5	111.50 ± 15.40 b	53.69 ± 29.32 b	106.503 ± 9.90 b
10	73.26 ± 11.23 c	69.49 ± 6.28 b	26.883 ± 9.39 d

[a] Results are expressed as means ± standard deviations of means (*n* = 3). Values followed by the same letter in the same column are not significantly different (*P* > 0.05).

cantly upon oxidation, except for the corn starch sample treated with the highest OGT. Oxidized tapioca starch exhibited a significant difference in pasting temperature after 1 and 3 min OGT, but the pasting temperature increased at the highest OGT. The reduction of pasting temperature following oxidation is a consequence of structural weakening and disintegration during oxidation (*22*). Peak viscosity for oxidized corn starches decreased significantly, whereas that of oxidized sago starch decreased markedly at 1 and 3 min OGT and significantly increased at 5 and 10 min OGT. In contrast, oxidized tapioca starch showed a significant increase in peak viscosity at 1 and 3 min OGT, but it considerably decreased at 10 min OGT. Slightly oxidized tapioca starches (1 and 3 min OGT) showed a significant increase in peak viscosity and final viscosity, which could be attributed to a cross-linking effect. This result agrees with that of Wang and Wang (*26*), who found that the increase in peak viscosity in slightly oxidized starch was due to the chemical cross-linking. Wongsagon et al. (*29*) also found that the increase in peak viscosity in periodate-oxidized tapioca starch was due to chemical cross-linking. Similarly, Jyothi et al. (*30*) observed that at low level of cross-linking, the peak viscosity of the cross-linked cassava starch increased in comparison to that of the native starch. The decrease in peak viscosity for oxidized starches could be due to partial cleavage of the glycosidic linkages after treatment with gaseous ozone, resulting in a decrease in the molecular weight of starch molecules. Thus, a partially degraded network would not be resistant to shear and could not maintain the integrity of the starch granule, thereby producing a lower viscosity. This scenario is supported by results reported by Sandhu et al. (*31*).

The breakdown viscosity is the difference between the peak viscosity and the hot paste viscosity. Higher breakdown viscosity indicates granule disruption or a lesser affinity of starch to resist shear force during heating. Breakdown viscosity for all oxidized corn and tapioca starches decreased considerably, whereas the reverse occurred for oxidized sago starch. The reduction in breakdown viscosity of oxidized corn and tapioca starches resulted from the introduction of new substituent groups into the oxidized starches (*23*). The Pearson correlation analysis of oxidized corn starch data shows a significant negative correlation between carboxyl content and breakdown viscosity (*r* = −0.862, *P* < 0.01). It appears that there was no direct correlation between breakdown and carboxyl content because some samples showed positive correlation but other samples showed negative correlation in Pearson correlation analysis. It seems that carboxyl content is not the main factor affecting breakdown viscosity. It could be due to the effect of ozone on the degradation of the amorphous region of the starch granules.

The increased peak viscosity for ozone-treated sago starch at 5 and 10 min OGT is related to the significant increase in carboxyl content at these times (**Table 2**). This result agrees with the finding by An and King (*32*), who found that the increase in viscosity was thought to be due to carboxyl groups formed during oxidation,

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Table 6. Pasting Properties of Ozone-Oxidized Starches[a]

sample		peak viscosity (RVU)	hot paste viscosity (RVU)	breakdown	cold paste viscosity (RVU)	setback (RVU)	pasting temp (°C)
corn	unmodified	249.17 ± 0.18 a	175.53 ± 0.27 a	73.44 ± 0.19 a	272.16 ± 0.10 a	96.39 ± 0.14 a	76.66 ± 0.05 a
	1 min	229.80 ± 0.11 b	171.88 ± 0.66 b	58.06 ± 0.82 b	247.03 ± 0.56 b	75.35 ± 0.99 b	76.02 ± 0.69 b
	3 min	216.33 ± 2.22 c	158.80 ± 1.20 c	57.49 ± 0.99 b	228.14 ± 1.50 c	69.30 ± 0.33 c	76.26 ± 0.34 ab
	5 min	204.72 ± 1.01 d	149.91 ± 1.41 d	54.51 ± 0.29 c	217.06 ± 0.75 d	67.05 ± 0.70 d	75.78 ± 0.10 b
	10 min	184.22 ± 0.91 e	134.52 ± 0.82 e	50.44 ± 1.59 d	193.25 ± 2.18 e	59.28 ± 1.97 e	76.61 ± 0.05 a
sago	unmodified	96.60 ± 0.25 c	41.24 ± 1.22 c	55.43 ± 0.87 d	62.77 ± 0.66 c	21.71 ± 0.49 c	77.29 ± 0.37 a
	1 min	97.68 ± 0.06 c	35.88 ± 0.79 d	61.98 ± 0.79 c	58.52 ± 0.97 d	22.31 ± 0.50 c	76.05 ± 0.18 c
	3 min	90.48 ± 0.25 d	28.65 ± 0.27 e	61.62 ± 0.29 c	44.95 ± 0.14 e	16.04 ± 0.19 d	76.50 ± 0.23 b
	5 min	232.49 ± 1.78 b	49.68 ± 0.60 b	183.71 ± 2.37 b	97.09 ± 0.23 b	47.78 ± 0.84 a	75.11 ± 0.01 d
	10 min	254.33 ± 1.70 a	55.91 ± 0.84 a	198.45 ± 1.08 a	99.13 ± 1.20 a	43.11 ± 0.44 b	75.91 ± 0.02 c
tapioca	unmodified	396.48 ± 0.03 c	148.76 ± 0.01 e	247.74 ± 0.03 a	241.09 ± 0.01 c	92.33 ± 0.01 a	68.65 ± 0.02 b
	1 min	425.92 ± 3.04 a	201.32 ± 4.93 a	226.63 ± 8.33 bc	272.97 ± 4.35 a	70.51 ± 8.07 b	68.53 ± 0.02 d
	3 min	407.03 ± 0.43 b	185.34 ± 2.27 b	220.85 ± 2.83 c	260.04 ± 1.17 b	74.53 ± 3.38 b	68.60 ± 0.00 c
	5 min	398.34 ± 0.08 c	167.84 ± 1.33 c	230.83 ± 1.41 b	227.60 ± 2.30 d	57.52 ± 4.54 c	68.63 ± 0.02 bc
	10 min	379.19 ± 1.85 d	160.00 ± 1.13 d	221.46 ± 0.83 c	205.38 ± 1.16 e	45.47 ± 0.17 d	68.68 ± 0.02 a

[a] Results are expressed as means ± standard deviations (*n* = 4). Values followed by the same letter in the same column are not significantly different (*P* > 0.05).

which may have kept the starch molecules from associating to allow more water uptake by the granules during heating. The increased breakdown viscosity of oxidized sago starch could be attributed to the weakened structure of the granules after treatment with gaseous ozone, which facilitated disruption of the granular structure. An and King (*32*) have reported that ozonation has increased breakdown viscosity of rice starch.

The setback viscosity indicates the degree of retrogradation of starch, mainly amylose (*33*); a high setback value indicates a high tendency of starch to retrograde. Data in **Table 6** show that, except for sago starch, the setback viscosity decreased progressively and significantly as oxidation level increased. When hot pastes are cooled, the extent of the increase in viscosity is governed by the reassociation tendency of the starch. This phenomenon is largely determined by the affinity of hydroxyl groups of one molecule for another. Oxidized starches, having been subjected to the introduction of carboxyl groups, were less prone to such reassociation. Introduction of carboxyl groups to replace the hydroxyl groups limits the formation of such binding forces. This accounts for the reductions in the setback of oxidized corn starch. This result agrees with Kuakpetoon and Wang's (*2*) report that amylose was likely excessively degraded in oxidized corn starches, resulting in lower final and setback viscosities. The presence of carbonyl and carboxyl groups caused by oxidation also impeded associations among starch chains, and a true gel structure was not obtained (*34*). These results suggest that differences in the type of starches used affected their responses to oxidation. The Pearson correlation analysis of oxidized corn and tapioca starch data shows a significant negative correlation between carboxyl content and setback (corn, $r = -0.833$; tapioca, $r = -0.897$, $P < 0.01$) and between carboxyl content and cold paste viscosity (corn, $r = -0.841$; tapioca, $r = -0.966$, $P < 0.01$). On the other hand, oxidized sago starch data in Pearson correlation analysis show a significant positive correlation between carboxyl content and setback ($r = 0.948$, $P < 0.01$) and between carboxyl content and cold paste viscosity ($r = 0.904$, $P < 0.01$). There is no direct correlation between carboxyl content and pasting properties. Corn starch seemed to be more susceptible to oxidation, with more significant reduction in pasting viscosity as compared with sago and tapioca starches at the same oxidation level. The Pearson correlation analysis for oxidized corn and tapioca starch data shows a significant negative correlation between the amount of ozone reacted with starch and pasting viscosity (corn, $r = -0.951$; tapioca, $r = -0.937$, $P < 0.01$). On the other hand, Pearson correlation analysis data show a significant positive correlation for oxidized sago starch ($r = 0.738$, $P < 0.01$). The higher

susceptibility of corn starch to oxidation could be due to the presence of natural pores in starch granules that allows ozone gas to diffuse into the granule.

In conclusion, gaseous ozone successfully oxidized the three types of starches studied, but the oxidation mechanism appeared to differ from that of chemical oxidation. Ozone oxidation introduced more carbonyl groups than carboxyl groups in the starch granules. Under identical conditions, the extent of starch oxidation varied among different types of starch.

Using ozone as an oxidizing agent offers several advantages over hypochlorite. During ozone oxidation, starch is oxidized in a dry state and in the "as is" moisture content condition, whereas hypochlorite oxidation requires use of organic solvents. The dry oxidation process using ozone is simpler than the hypochlorite oxidation process, and it offers 100% product recovery as compared to hypochlorite oxidation, which inevitably suffers some losses during filtration after the oxidation process.

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