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Characterization of physicochemical properties of hypochlorite- and peroxide-oxidized cassava starches

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ABSTRACT

Physicochemical properties of oxidized cassava starch as influenced by different oxidizing agents (sodium hypochlorite and hydrogen peroxide) were investigated. The starch was modified under controlled temperature and pH with 3% oxidants (based on starch) between 30 and 300 min. The results showed that hypochlorite oxidation favored the formation of carboxyl group while carbonyl was the major functional group formed during peroxide oxidation. The molecular sizes determined by HPSEC and the apparent viscosity of starch were decreased to a similar extent by both oxidants; however, the changes observed in peroxide oxidation proceeded with a faster rate. Rheological measurement revealed that peroxide-oxidized starches had a higher tendency for gelation and gave a firmer gel than hypochlorite-oxidized starches. The gelatinization temperatures increased following peroxide oxidation but decreased after hypochlorite oxidation. The gelatinization enthalpy decreased after oxidation by both chemicals. Results from DSC demonstrated that amylopectin retrogradation was not significantly affected by both types of oxidation.

1. Introduction

Oxidized starch is widely used in many industries, particularly in applications where film formation and adhesion properties are desired. The major application of oxidized starch is as surface sizing agents and coating binders in the paper industry. Oxidized starch is commonly produced by reaction of starch with an oxidizing agent under controlled temperature and pH. During oxidation process, hydroxyl groups on starch molecules are oxidized to carbonyl and carboxyl groups, contributing improved stability to starch paste. The reaction also causes degradation of starch molecules resulting in a modified starch with low viscosity. This allows the use of oxidized starch in application where high solid concentration is needed (Wurzburg, 1986).

Several oxidizing agents have been applied to starch oxidation including sodium hypochlorite, bromine, periodate, permanganate, hydrogen peroxide and ammonium persulfate. Among them hypochlorite oxidation is the most common method for the production of oxidized starch in an industrial scale. This oxidant is efficient but may lead to a formation of toxic chlorinated by-products. Hydrogen peroxide, an alternative oxidizing agent, has been used in a commercial practice to a much lesser extent. Unlike sodium hypochlorite, hydrogen peroxide creates no harmful by-product. Hydrogen peroxide decomposes inevitably to oxygen and water (Isbell & Frush, 1987). This chemical is therefore considered more environmentally friendly and is preferred especially when a chlorine-free process is desired (Ketola & Hagberg, 2003).

Oxidation causes various alterations to starch molecular structures resulting in modified starches with different characteristics. The extent of changes on the structural and physicochemical properties of oxidized starch depends mainly on the botanical origin of native starch, the type of oxidizing agent and the reaction conditions. Many studies on starch oxidation by sodium hypochlorite have been reported. Tuber starches were found to be more readily oxidized than cereal starches (Forssell, Hamunen, Autio, Suortti, & Poutanan, 1995; Kuakpetoon & Wang, 2001). Amylose content of native starch was suggested to play a significant role in controlling the efficiency of hypochlorite oxidation (Kuakpetoon & Wang, 2006). Oxidation of starch with different levels of sodium hypochlorite has been shown to yield oxidized products with different pasting characteristics (Sangseethong & Sriroth, 2002; Wang & Wang, 2003). The rate of oxidation reaction as well as the type and amount of functional groups formed in the starch molecules during hypochlorite oxidation was reported to depend on the reaction pH (Hullinger & Whistler, 1951; Patel, Mehta, & Srivastava, 1974;

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Sangseethong, Lertphanich, & Sriroth, 2009; Schmorak & Lewin, 1963; Schmorak, Mejzler, & Lewin, 1961; Whistler & Schweiger, 1957).

Relatively few studies on starch oxidation by hydrogen peroxide are available in the literature. In the oxidation of amylopectin with high level of hydrogen peroxide, Whistler and Schweiger (1959) observed an extensive degradation of amylopectin at reaction pH higher than 7 whereas little oxidation occurred at reaction pH lower than 5. Parovuori, Hamunen, Forssell, Autio, and Poutanen (1995) demonstrated the influence of different metal catalysts on peroxide oxidation of potato starch. Wing and Willett (1997) reported the use of a reactive extrusion process to produce water-soluble oxidized starch by hydrogen peroxide.

Studies on comparison of oxidized starches prepared by different oxidants are very scarce. Only a few studies on the structural characteristics and substitution pattern of potato starch oxidized by sodium hypochlorite and hydrogen peroxide have been reported (Manelius, Buleon, Nurmi, & Bertoft, 2000; Zhu & Bertoft, 1997; Zhu, Sjoholm, Nurmi, & Bertoft, 1998). The aim of this study was to compare the physicochemical and functional properties of hypochlorite- and peroxide-oxidized starches. Cassava starch was oxidized with sodium hypochlorite and hydrogen peroxide at varying reaction times. The physicochemical properties of the resulting oxidized starches including the formation of carbonyl and carboxyl groups, molecular size distribution, gelatinization, retrogradation and rheological properties were then evaluated and compared.

2. Materials and methods

2.1. Materials

Native cassava starch was obtained from Sanguan Wongse Industries Co., Ltd., Nakhonratchasima, Thailand. Sodium hypochlorite containing 10% active chlorine (w/w) was obtained from B.S. International Co., Ltd. (Thailand). All other chemicals used in the study were of analytical grade.

2.2. Preparation of oxidized starch

2.2.1. Sodium hypochlorite oxidation

Hypochlorite-oxidized cassava starch was prepared as previously described (Sangseethong et al., 2009) with some modifications. A cassava starch slurry containing 40% dry solid was prepared and the pH was adjusted to 10 with NaOH solution. The temperature of the slurry was maintained at 30 °C, and sodium hypochlorite (3% active chlorine based on starch) was added dropwise over a period of 15 min with stirring. During the addition of reagent and the course of reaction, pH of the slurry was maintained at 10 with NaOH solution. The mixture was stirred under the defined conditions and the sample was collected at the reaction time of 30, 60, 120 and 300 min (the time was counted after all reagent was added). The reaction in the collected samples was terminated by addition of sodium bisulphite and the pH was adjusted to 6.5–7.0. The sample was then filtered and thoroughly washed with water until the filtrate gave negative response to silver nitrate solution. The obtained starch was then dried in an oven at 50 °C.

2.2.2. Hydrogen peroxide oxidation

Hydrogen peroxide oxidation of starch was carried out as described by Parovuori et al. (1995) with some modifications. A cassava starch slurry containing 40% dry solid was prepared and the pH was adjusted to 10 with NaOH solution. The temperature of the slurry was maintained at 30 °C. Copper sulfate (0.1% based on starch) was added as a catalyst and hydrogen peroxide solution was added dropwise over a period of 15 min to the reaction mixture to reach a final concentration of 3% (based on starch). During the addition of reagent and the course of reaction, pH of the slurry was maintained at 10 with NaOH solution. The sample was collected at the reaction time of 30, 60, 120 and 300 min (the time was counted after all reagent was added). The collected sample was treated in the same manners as described above (in Section 2.2.1).

2.3. Morphology of starch granule

Granule morphology of starch samples was studied with a scanning electron microscope (SEM) (JEOL JSM-5310, England). Starch samples were mounted on circular aluminum stubs with double sticky tape, coated with gold and then examined at an acceleration voltage of 10 kV.

2.4. Determination of carbonyl content

The carbonyl content was determined as described by Kuakpetoon and Wang (2001). Starch sample (4 g) was slurried in 100 mL of distilled water. The slurry was gelatinized in a boiling water bath for 20 min, cooled to 40 °C and adjusted to pH 3.2 with 0.1 M HCl. Then 15 mL of hydroxylamine reagent was added. The flask was stoppered and agitated in a water bath at 40 °C. After 4 h, the sample was rapidly titrated to pH 3.2 with 0.1 M HCl. A blank determination with only hydroxylamine reagent was performed in the same manner. The hydroxylamine reagent was prepared by dissolving 25 g hydroxylamine hydrochloride in 100 mL of 0.5 M NaOH. The final volume was then adjusted to 500 mL with distilled water.

2.5. Determination of carboxyl content

The carboxyl content of starch was determined following the FAO method (2001) with some modifications. Starch sample (5 g) was stirred in 25 mL of 0.1 M HCl for 30 min. The slurry was then filtered and washed with distilled water until free of chloride ions. The filtered cake was transferred to a 600 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 sample was immediately titrated with 0.1 M NaOH using phenolphthalein as indicator. A blank determination was run on the original sample in the same manner but being stirred in 25 mL of distilled water instead of 0.1 M HCl.

2.6. Molecular size distribution

The molecular size distributions of native and oxidized starches were determined by High Performance Size Exclusion Chromatography (HPSEC) (Shimadzu, Tokyo, Japan). Starch (40 mg) was dispersed in 9 mL of deionized water and heated in boiling water for 20 min. After cooling to room temperature, 1 mL of 1.0 M $NaNO_3$ was added and the sample was filtered through an 8.0 μm filter. The filtrate was then injected into the HPSEC system consisting of a LC-10AT pump, SIL-10A automatic injector, RID-10A refractive index detector and three Ultrahydrogel columns (Ultrahydrogel linear, Ultrahydrogel 120 and Ultrahydrogel 120) connected in series. Columns were maintained at 55 °C and RI detector at 80 °C. The mobile phase was 0.1 M $NaNO_3$ at a flow rate of 0.3 mL/min.

2.7. Viscosity and viscoelastic properties of starch dispersion

The measurements of viscosity and viscoelastic properties of oxidized starch pastes were performed on a rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) using a concentric cylinder (diameter of cup and bob, 28.92 and 26.66 mm, respectively). The temperature was regulated by a

Paar Physica circulating bath and a controlled Peltier system (TEZ 150P/MCR) with an accuracy of ±0.1 °C. The starch paste was prepared by heating 15% (w/w) of starch slurry in a water bath at 95 °C for 15 min using an overhead stirrer set at 200 rpm. During heating, the sample jar was covered with a lid to avoid water loss. For viscoelastic measurement, the hot paste was immediately transferred to the sample cup preheated to 90 °C. While the paste was cooled to 20 °C at a rate of 1.5 °C/min, the storage (G') and loss (G'') moduli were measured with a small amplitude oscillatory test at a frequency of 10 rad/s. The measurements were performed at a strain of 1%, which was within the linear viscoelastic region. Apparent viscosity of starch paste was measured over a shear rate range of 0.1–500 s^{-1} at 80 °C. The viscosity values at the shear rate of 22 s^{-1} were used for comparison between different samples.

2.8. Gelatinization and retrogradation properties by DSC

The gelatinization and retrogradation properties of the starch samples were measured using a Perkin-Elmer Differential Scanning Calorimeter (DSC7, Norwalk, CT). Starch was weighed into a stainless steel DSC pan and deionized water was added to give 70% moisture content. The pan was sealed, equilibrated at room temperature overnight, and scanned from 0 to 120 °C at a rate of 10 °C/min. After scanning, the gelatinized sample was stored at 4 °C for 7 days, after which the sample was left at room temperature for 1 h and rescanned under the same conditions with the first scanning. An empty pan was used as the reference and the DSC was calibrated with indium. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and the enthalpies of gelatinization (ΔH_g) and retrogradation (ΔH_r) were determined.

3. Results and discussion

3.1. Morphology of starch granules

Scanning electron micrographs of native and oxidized cassava starch granules are shown in Fig. 1. Native cassava starch granules had round shape with a truncated end on one side. The surface of native starch granules was smooth with no evidence of any fissures (Fig. 1a). In general, similar pattern of changes on external morphology of starch granules was observed for oxidized starches produced by either hypochlorite or peroxide oxidation. There were no obvious changes or signs of damages on the granule surface of oxidized starch with 30 min of reaction time (Fig. 1b and f). However, after 60 min, a slightly roughened surface was observed (Fig. 1c and g). With the oxidation time of 120 and 300 min, the granule surface became rougher. Fissures could be observed on the surface of some starch granules (directed by arrows in Fig. 1d, e, h and i), presumably due to a localized extensive oxidation. Rutenberg and Solarek (1984) reported that the surfaces of corn and potato starch granules were unaffected by hypochlorite oxidation up to 6% active chlorine while Kuakpetoon and Wang (2001) observed no changes on granule morphology of potato, corn and rice starches modified with hypochlorite at the levels of 0.8 and 2% active chlorine. The results observed in our hypochlorite-oxidized starches differs from the previous reports are probably due to the differences in the granular architecture and fragility of starch granules from various botanical sources as well as the different reaction conditions employed in the oxidation process.

3.2. Carbonyl and carboxyl contents

The relationship between the formation of carbonyl and carboxyl groups during starch oxidation is not yet completely understood. Previous studies have proposed a consecutive reaction path in which hydroxyl groups in starch molecules are first oxidized to carbonyl groups and then to carboxyl groups (Kuakpetoon & Wang, 2006; Whistler & Schweiger, 1957). However, depending on the type of oxidant used and the reaction conditions, parallel reaction paths in which carbonyl or carboxyl groups are selectively formed by oxidation of the hydroxyl groups at the certain positions on glucosidic ring are also reported (Bragd, Besemer, & van Bekkum, 2000).

The carbonyl and carboxyl contents of hypochlorite- and peroxide-oxidized cassava starches obtained from this study are shown in Fig. 2. In hypochlorite oxidation, the major functional group produced was carboxyl which progressively increased with reaction time while only minor amount of carbonyl groups was formed and its content stayed relatively unchanged with reaction time (Fig. 2a). This is in agreement with the earlier works reporting that hypochlorite oxidation of starch when being performed under alkaline conditions favors the formation of carboxyl groups (Wurzburg, 1986). The pattern of carbonyl and carboxyl group formation as a function of reaction time supports that reaction path of hypochlorite oxidation is consecutive with carbonyl groups as intermediates, which are further oxidized to carboxyl groups as the primary final product. The results suggest that the conversion from carbonyl to carboxyl groups was very fast. The initial steps prior to the formation of carbonyl groups might probably be the rate limiting step; however, once carbonyl groups were formed, they were rapidly converted to carboxyl groups. Therefore, no substantial amount of carbonyl groups was accumulated over the entire period of reaction time.

In contrast to hypochlorite oxidation, carbonyl was a primary functional group produced in the peroxide-oxidized starches whereas a minor amount of carboxyl groups was formed (Fig. 2b). The reaction mechanisms of hydrogen peroxide with starch are very complex and have been reported to proceed via a radical chain reaction. In the presence of metal catalyst, hydrogen peroxide is rapidly decomposed affording hydroxyl radical ($HO^{\bullet}$). This highly reactive free radical readily reacts with carbohydrate by abstracting hydrogen from a C–H group on the sugar ring, forming a radical ($R^{\bullet}CHOH$) which further undergoes acid or base catalyzed rearrangement resulting in cleavage of glycosidic bond and a carbonyl group (Arts, Mombarg, van Bekkum, & Sheldon, 1997). Under alkaline conditions, carbohydrates having a free or potentially free carbonyl group could undergo further reactions via various pathways; some of which afford a carboxyl group (Isbell & Frush, 1987). Being highly reactive, hydroxyl radical has been reported to react with carbohydrate exceedingly rapidly (Fry, 1998). This probably explains the pattern of functional group formation observed in Fig. 2b. It is speculated that in our study hydrogen peroxide reacted with starch so rapidly that most reagent was consumed for hydrogen abstraction affording high amount of carbonyl groups during the early period of reaction time; thus, a lot less oxidant was available for oxidation of carbonyl to carboxyl groups. Results in Fig. 2b showing that carbonyl and carboxyl contents varied very little with reaction time between 30 and 300 min also support that starch oxidation by peroxide was very rapid. It seems that oxidation of hydroxyl groups in starch molecules by peroxide was almost completed within the first 30 min.

3.3. Molecular size distribution

The molecular size distributions of native and oxidized cassava starches analyzed with HPSEC are presented in Fig. 3. The first fraction of the native cassava starch with a shorter retention time consisted of high molecular weight carbohydrates, mainly amylopectin. The second fraction appeared as a shoulder of the first peak was composed of low molecular weight carbohydrates, mainly amylose. After oxidation, it was observed that area of the first frac-

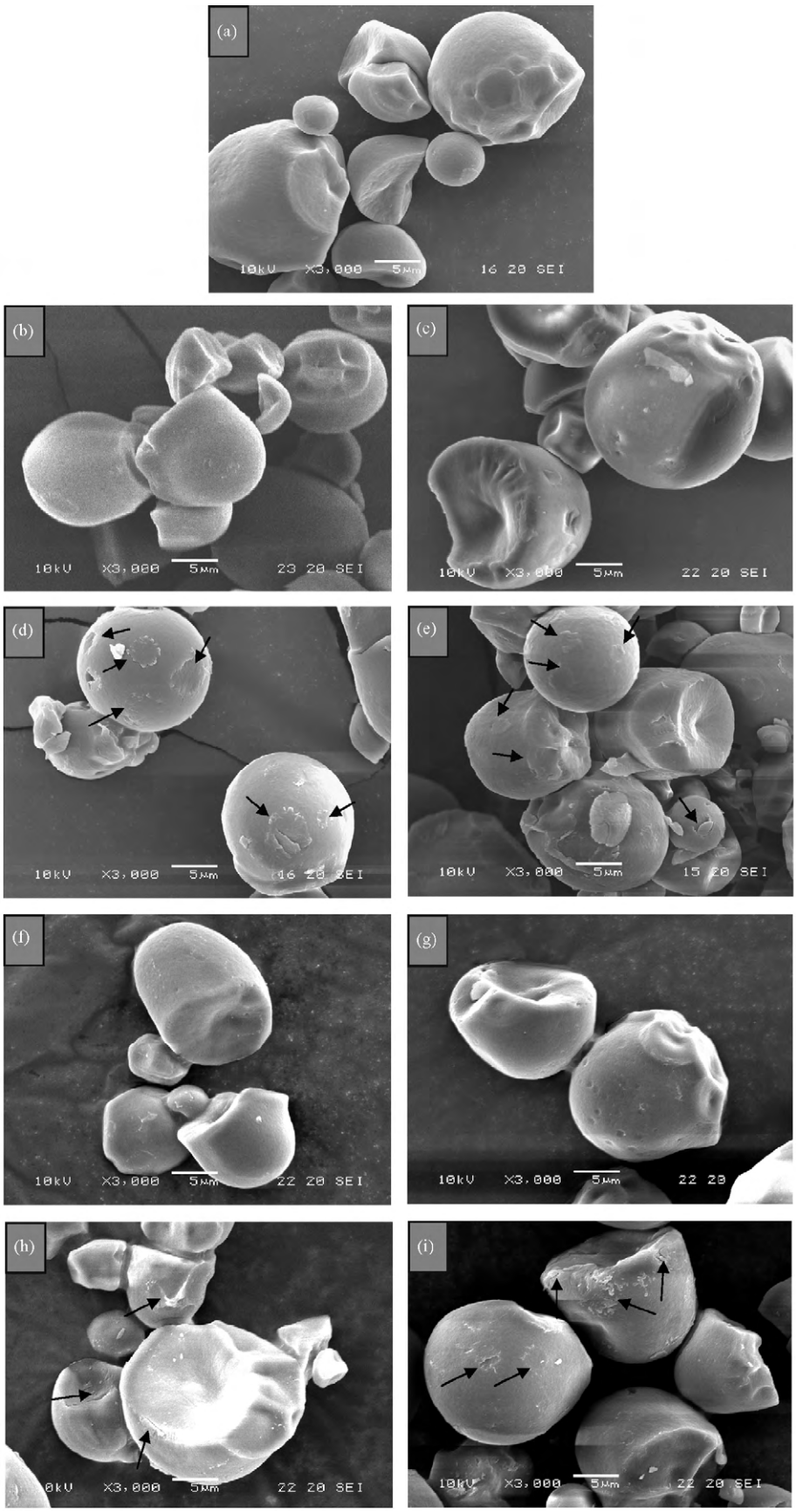

Fig. 1. Scanning electron micrographs of native (a) and oxidized cassava starches prepared with hypochlorite (b–e) and peroxide (f–i) at various reaction times: 30 min (b and f), 60 min (c and g), 120 min (d and h) and 300 min (e and i).

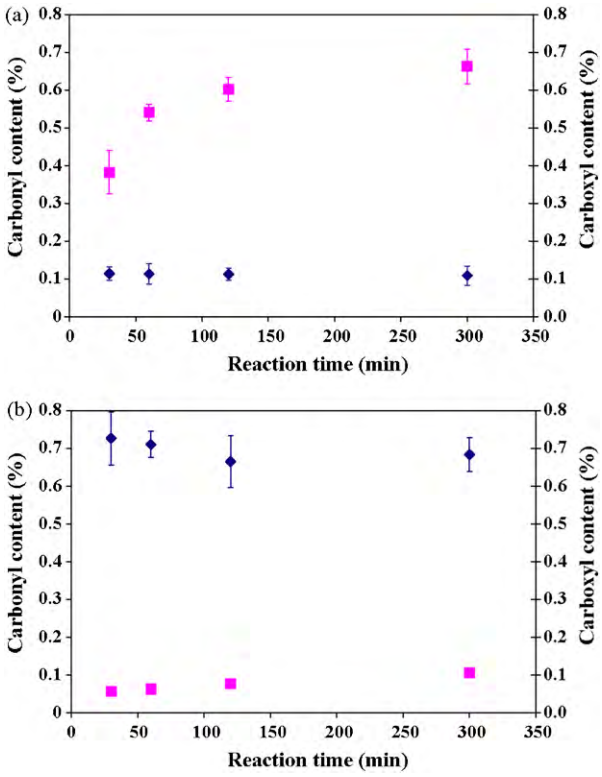

Fig. 2. Carbonyl (♦) and carboxyl (■) contents of oxidized cassava starches prepared at various reaction times: (a) hypochlorite oxidation; (b) peroxide oxidation.

tion decreased while areas of fractions with the longer retention time increased. The results indicated that oxidative treatment by either hypochlorite or peroxide caused depolymerization of amylopectin to the lower molecular weight molecules. For samples

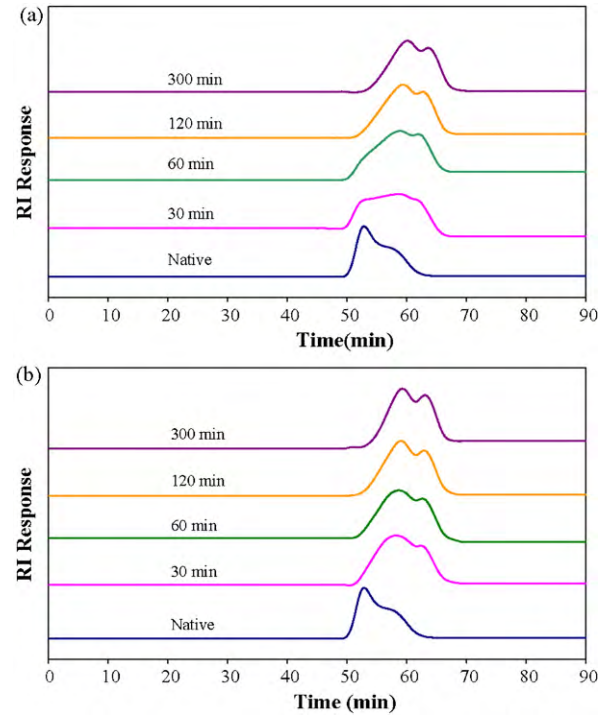

Fig. 3. Molecular size distributions of native and oxidized cassava starches. The number on each curve represents the reaction time: (a) hypochlorite oxidation; (b) peroxide oxidation.

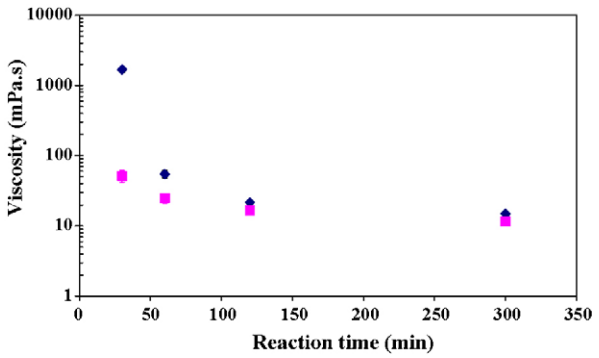

Fig. 4. Viscosity of oxidized cassava starches prepared with hypochlorite (♦) and peroxide (■) at various reaction times. Viscosity of 15% starch paste measured at a shear rate of 22 s^{-1} and a temperature of 80 °C.

with the shorter reaction time, it was apparent that the extent of decreasing in the area of the first peak in peroxide oxidation was higher than that of the hypochlorite oxidation, indicating that peroxide oxidation caused higher depolymerization of the amylopectin molecules than hypochlorite oxidation. This confirms the lower viscosity of peroxide-oxidized starches than hypochlorite-oxidized starches observed in samples with the shorter reaction time, particularly at 30 and 60 min (Fig. 4).

3.4. Hot paste viscosity

The apparent viscosity of oxidized starch was measured in a 15% suspension and at 80 °C. Under these conditions, the paste from native cassava starch was too viscous to measure; hence its viscosity was not determined. The viscosity of oxidized starches decreased with increasing reaction time (Fig. 4). The decrease in viscosity of hypochlorite- and peroxide-oxidized starches can be attributed to the oxidative cleavage of starch chains, resulting in starch with the lower molecular size (Kuakpetoon & Wang, 2001; Rutenberg & Solarek, 1984). The rate of viscosity reduction seemed to be faster in peroxide oxidation. At 30 min reaction time, the viscosity of hypochlorite-oxidized starch was 1685 mPa s while peroxide-oxidized starch was only 51 mPa s. However, at longer reaction times the viscosity of both oxidized starches was in the similar range.

In addition to oxidizing hydroxyl groups to carbonyl and carboxyl groups, oxidative treatment is also capable of cleaving carbohydrate chains. The scission of carbohydrate chains may or may not have proceeded independently of the functional group formation. In hypochlorite oxidation, the increase in carboxyl content (Fig. 2a) and a decrease in starch viscosity (Fig. 4) seemed to occur simultaneously with a similar pattern; being faster in the first 60 min and slower thereafter. This probably implies that the scission of starch chains in hypochlorite oxidation occurred concomitantly with the formation of functional groups (i.e. carboxyl groups in this case). It has been proposed that hypochlorite preferentially oxidizes hydroxyl groups at C2 and/or C3 of anhydroglucose units to carbonyl groups and eventually to carboxyl groups. The presence of oxidized groups at C2 or C3 would weaken the bond at the C1 position, leading to depolymerization of starch chains via β-elimination (Whistler, Linke, & Kazeniac, 1956) which would occur swiftly under alkaline conditions used in this study. From our study it appeared that carbonyl groups stayed relatively constant whereas carboxyl groups increased with a concurrent decrease in starch viscosity (Figs.2a and 4). These results imply that in hypochlorite oxidation, the initial step in oxidizing hydroxyl to carbonyl group is probably a rate limiting step (as earlier discussed in Section 3.2). Once the carbonyl group was formed, it was rapidly

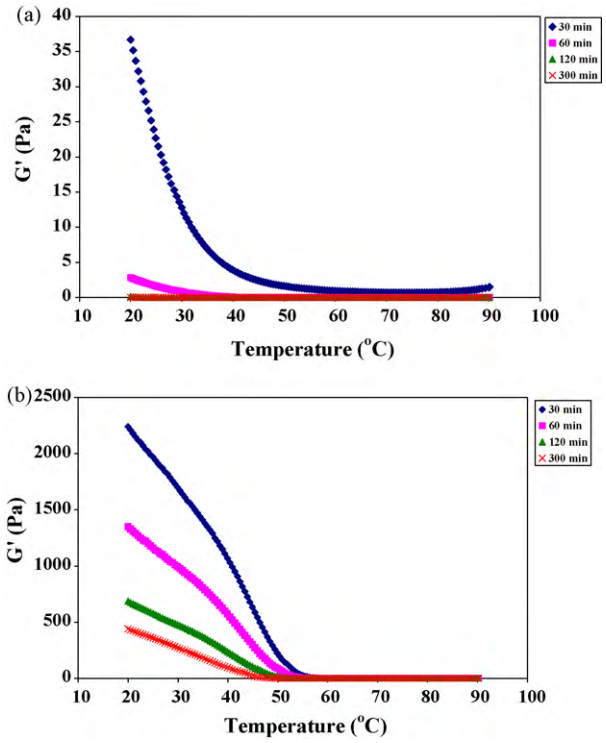

Fig. 5. Changes in storage modulus (G') of 15% oxidized cassava starch paste during cooling from 90 to 20 °C. Values in the legend box represent the reaction time of starch modification: (a) hypochlorite oxidation; (b) peroxide oxidation.

oxidized to carboxyl group and the starch chain was simultaneously cleaved via a β-elimination. However, this cannot rule out the existence of other reaction paths in which functional group formation and/or chain cleavage might independently take place.

In peroxide oxidation, the reactions proceeded at a relatively faster rate. Both carbonyl and carboxyl contents of peroxide-oxidized starches remained practically unchanged while only a slight decrease in starch viscosity was observed between the reaction time of 30–300 min (Figs.2b and 4). It seemed that the reactions causing structural changes (i.e. functional group formation and molecular size reduction) of starch molecules during peroxide oxidation were mostly occurred within the first 30 min. As a result, it may not be possible to determine how the scission of starch chains would relate to the functional group formation from the data obtained in this study. The reactions probably proceeded via a previously proposed mechanism in which the rearrangement of a free radical (R•CHOH) leads to a cleavage of glycosidic bond and the formation of a carbonyl group (Arts et al., 1997). A slight decrease in starch viscosity observed between 30 and 300 min probably implies that there might be the scission of starch chains during this time range which possibly proceeded independently of the functional group formation.

3.5. Rheological properties

Changes in storage moduli (G') during cooling from 90 to 20 °C for both hypochlorite- and peroxide-oxidized cassava starches are shown in Fig. 5 (loss moduli, G'', was also monitored but it is not shown in Fig. 5 to improve clarity). At high temperature, all samples exhibited fluid-like character ($G'' > G'$). Cooling starch pastes from 90 to 20 °C increased both G' and G'' and in some samples a crossover of G' and G'' around 40–60 °C (data not shown) was observed indicating a gelling point. The sharp increase in G' (as observed in Fig. 5) is attributed to the onset of intermolecular association of starch molecules leading to the development of a gel network structure (Chang, Lim, & Yoo, 2004; Nickerson, Paulson, & Speers, 2004).

The rate of gel formation and the rigidity of the gels varied considerably between samples. It seems that both gel formation and gel rigidity in each group of oxidized starches decreased with an increase in degree of oxidation. The reason for this could be due to the structural changes brought to starch molecules during oxidation reactions, which are depolymerization and the formation of carbonyl and carboxyl groups. Starch with smaller molecular size may contribute to less entanglement and probably leading to a smaller and weaker junction zone while the presence of both functional groups may hinder the re-association of amylose chains. Results in Fig. 5 indicated that hypochlorite-oxidized starches had a lower tendency for gel formation. Only sample with the shortest reaction time (30 min) of hypochlorite-oxidized starches formed a gel (Fig. 5a) whereas gel formation could be observed in all samples of peroxide-oxidized starches (Fig. 5b). In addition, the gel of hypochlorite-oxidized starch was much weaker than those of peroxide-oxidized starches as indicated by the magnitude of G' at the end of cooling period (being 37 Pa and 440–2240 Pa for hypochlorite- and peroxide-oxidized starches, respectively). The differences in the gelling behaviors observed between these two groups of oxidized starches may be due to the presence of relatively high amounts of carboxyl groups in hypochlorite-oxidized starches. The carboxyl groups carrying negative charges seemed to be more effective in hindering interchain association of amylose molecules and minimizing junction zone formation than the carbonyl groups found in high amount in peroxide-oxidized starches.

3.6. Gelatinization properties

The transition temperatures (T_o, T_p and T_c) and enthalpies (ΔH_g) associated with gelatinization of native and oxidized cassava starches are presented in Table 1. Compared to native starch, onset temperature of gelatinization (T_o) reduced in hypochlorite-oxidized starches (1–3 °C lower than native starch) but increased in peroxide-oxidized starches (3–6 °C higher than native starch). The

Table 1
Gelatinization properties of native and oxidized cassava starches.

Starch sample	Reaction time (min)	Gelatinization temperature			Gelatinization enthalpy (ΔH_g) (J/g)
		T_o (°C)	T_p (°C)	T_c (°C)	
Native	–	64.3 ± 0.0	71.7 ± 0.0	81.0 ± 0.0	17.4 ± 0.1
NaOCl-oxidized	30	62.9 ± 0.2	70.9 ± 0.2	85.4 ± 1.0	16.6 ± 0.2
	60	61.9 ± 0.3	68.9 ± 0.4	88.4 ± 0.2	16.2 ± 0.5
	120	61.7 ± 0.4	69.2 ± 0.4	88.8 ± 0.3	15.6 ± 0.2
	300	61.6 ± 0.2	68.9 ± 0.0	89.9 ± 0.4	14.1 ± 0.0
H_2O_2-oxidized	30	67.4 ± 0.0	74.7 ± 0.2	90.2 ± 0.7	15.6 ± 0.1
	60	69.0 ± 0.1	75.9 ± 0.1	92.9 ± 0.3	15.8 ± 0.1
	120	69.8 ± 0.1	76.5 ± 0.3	95.0 ± 0.3	15.1 ± 0.1
	300	70.4 ± 0.2	77.2 ± 0.1	96.8 ± 0.5	14.9 ± 0.1

Table 2
Retrogradation properties of native and oxidized cassava starches.

Starch sample	Reaction time (min)	Transition temperature of retrograded sample			Retrogradation enthalpy (ΔH_r) (J/g)
		T_o (°C)	T_p (°C)	T_c (°C)	
Native	–	42.8 ± 0.3	53.9 ± 0.4	62.0 ± 0.1	6.1 ± 0.2
NaOCl-oxidized	30	42.2 ± 0.0	53.0 ± 0.0	62.9 ± 0.1	6.0 ± 0.0
	60	42.7 ± 0.2	52.8 ± 0.1	62.7 ± 0.0	6.3 ± 0.2
	120	42.6 ± 0.2	53.1 ± 0.2	62.6 ± 0.1	6.8 ± 0.2
	300	40.0 ± 1.9	51.5 ± 1.3	62.8 ± 0.8	6.7 ± 0.5
H_2O_2-oxidized	30	46.6 ± 0.1	56.1 ± 0.0	64.5 ± 0.3	6.3 ± 0.0
	60	41.0 ± 0.3	53.0 ± 0.4	62.9 ± 0.4	5.9 ± 0.1
	120	41.7 ± 0.3	53.3 ± 0.5	63.1 ± 0.1	5.8 ± 0.1
	300	42.5 ± 0.4	53.4 ± 0.5	63.1 ± 0.3	5.8 ± 0.1

different effects of oxidation on the gelatinization temperature of cassava starch when using hypochlorite as an oxidant as compared with peroxide implies that these two chemicals worked by different mechanisms.

Starch gelatinization is primarily a swelling driven process. It has been suggested that water uptake by the amorphous growth ring is accompanied by swelling within this region which imposes a stress upon crystalline domains and causes amylopectin double helices within the crystallites to dissociate (Jayakody & Hoover, 2002). The increased T_o observed in peroxide-oxidized starches was probably attributed to the depolymerization in the amorphous region which functions to destabilize the crystalline lamella. Once the amorphous regions were degraded, their destabilizing effect on the crystalline domains was destroyed. Therefore, the gelatinization of the resulting starch occurred at a higher temperature. A similar increase in T_o of gelatinization was also previously observed in the peroxide-oxidized potato starch (Manelius et al., 2000; Parovuori et al., 1995). On the other hand, hypochlorite-oxidized starches showed a decrease in T_o which was also observed in our previous study (Sangseethong et al., 2009). In addition to starch degradation, hypochlorite oxidation also introduced high amount of carboxyl groups into starch granules. The negatively charged carboxyl groups could readily adsorb water and facilitate hydration. This would weaken the starch granule and cause it to gelatinize at a lower temperature.

The enthalpies of gelatinization of both types of oxidized starches were decreased when compared to the native starch. This suggests that both hypochlorite and peroxide oxidation caused a weakening of starch granules, probably from the partial degradation of starch molecules in the crystalline lamella. Consequently, less energy was needed to gelatinize starch.

3.7. Retrogradation properties

The retrogradation properties of native and oxidized cassava starches were studied after storage of gelatinized starches at 4 °C for 7 days. The transition temperatures (T_o, T_p and T_c) and the melting enthalpies (ΔH_r) of retrograded starches as determined by DSC are summarized in Table 2. The endothermic transition observed during reheating of the stored starch paste reflects an overall measure of the ordered structure developed from recrystallization of amylopectin during cold storage (Karim, Norziah, & Seow, 2000). Recrystallization of starch molecules has been reported to occur in a less ordered manner than that in the original granules (Perera & Hoover, 1999). This explains the observation of retrogradation endotherms at a much lower temperature (40.0–64.5 °C as shown in Table 2) when compared to that of the gelatinization process (61.6–96.8 °C as shown in Table 1).

It has been suggested that oxidation could either increase or decrease the retrogradation of starch molecules via two different mechanisms. The degradation of long chain amylopectin or even amylose molecules in the amorphous lamellae could produce dextrins with an appropriate length for re-association which could promote starch retrogradation. On the contrary, the formation of carboxyl or carbonyl groups on oxidized starch molecules would hinder the chain re-association, resulting in a lower tendency for retrogradation (Kuakpetoon & Wang, 2006). These two mechanisms could concurrently take place in the starch granules during modification process; thus, the predominant mechanism would determine the net effect of oxidation on the starch retrogradation properties. This may explain the contradict observations previously reported in the literatures. Lawal, Adebowale, Ogunsanwo, Barba, and Ilo (2005) and Sandhu, Kaur, Singh, and Lim (2008) observed a decrease in the enthalpy of retrogradation of hypochlorite-oxidized starch while Kuakpetoon and Wang (2006) reported a slight increase. Data in Table 2, show that ΔH_r of both hypochlorite- and peroxide-oxidized starches was not significantly different from that of the native starch suggesting that oxidation by both reagents under the conditions used in this study had negligible effect on amylopectin retrogradation.

Unlike results from DSC, the study on the rheological behavior (gelation tendency) of starch paste during cooling (in Section 3.5) indicated that peroxide-oxidized starches had a higher degree of gel formation whereas hypochlorite-oxidized starches showed a lower tendency. The initial development of starch gelation has been suggested to be dominated by the formation of an amylose matrix gel (Miles, Morris, Orford, & Ring, 1985). The results from DSC and rheological studies may imply that structural alteration during oxidation might preferentially take place on the amylose fraction, thus leaving amylopectin retrogradation mostly unaffected as also suggested in other modifications (Tran, Piyachomkwan, & Sriroth, 2007). These results also support the previous study which reported that amylose was more susceptible to hypochlorite oxidation than amylopectin (Kuakpetoon & Wang, 2006; Wang & Wang, 2003).

4. Conclusions

The type of oxidizing agents used during the modification process significantly influenced the physicochemical properties of the resulting oxidized starches. Under the conditions used in this study, hypochlorite oxidation favored the formation of carboxyl group while carbonyl was a major functional group found in peroxide-oxidized starches. Both reagents could produce oxidized starches with similar range of viscosity and molecular size distribution but the changes observed in peroxide oxidation proceeded with a faster rate. Upon cooling, peroxide-oxidized starches tended to gel more readily and gave a firmer gel when compared to hypochlorite-oxidized starches. The gelatinization temperatures increased in peroxide-oxidized starches but decreased in hypochlorite-oxidized starches. DSC study revealed that both types of oxidation had negligible effect on amylopectin retrogradation suggesting that the oxidation may preferentially take place on the amylose fraction.

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