Physicochemical properties of ozone-oxidized cassava starch under different slurry concentration

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Abstract. Chemical reagents used for starch oxidation induces chemical waste problems and potentially leaves undesirable residues in the product. Ozone-oxidation is one of the advanced oxidation process (AOP) methods and the latest rapid oxidation process which is safe for food products so that ozone is considered to be a good alternative for oxidation. This work aimed to evaluate the changes of physicochemical properties of oxidized cassava starch under different concentrations of slurry and dissolved ozone. The physicochemical of cassava starch oxidized by dissolved ozone (0.8, 1.4, and 2.0 ppm) with different concentrations of slurry (1:6 and 1:10) were investigated. Ozone and concentration of slurry significantly affected the physicochemical properties of modified cassava starch. Carbonyl and carboxyl contents, swelling power, and solubility increased as ozone concentration increased up to 1.4 ppm. The ozone-oxidation significantly increased amylose content and paste clarity and decreased paste viscosity. Ozone concentration 1.4 ppm with a concentration of slurry 1:10 seemed to be the most favourable condition for the oxidation process.

Keyword: dissolved ozone, slurry, ozone-oxidation, oxidized starch, cassava starch

1. Introduction

Starch modification using physical, chemical, and enzymatic or combinations of some methods is carried out to obtain the desired characteristics of starch. Modified starch generally has new functional properties such as lower viscosity, higher stability, higher solubility and film formation, so that its application can be expanded. Modified starch provides functional properties which can be beneficial and add value to starch itself [1]. Chemical modification still be an option in starch modification because the process is easily controlled and the mechanism of chemical modification is relatively easy to understand compared to other starch modification methods [2].

Oxidation is one of starch modification methods which has several unique functional properties such as clarity, low viscosity, high stability, film-forming and binding properties [3]. Chemical oxidizing agents are likely to be undesirable for starch modification because of its safety and nutrition issue, for example, benzoyl peroxide and chlorine dioxide which will leave a residue of benzoic acid and chlorine respectively [4].

Ozone is a more powerful oxidant than common oxidizing agents, such as sodium hypochlorite and hydrogen peroxide. It is considered as a safe starch modification method for both consumers and the environment. In addition, ozone is a strong and environmentally friendly oxidizing agent compared to
other oxidizing agents. Ozone is easily degraded to oxygen so that it will not cause any problem in the environment and leaves no residue in the products [5]. Ozone-oxidation is a well-known technology and a strong oxidizing agent which can effectively oxidize many organic compounds in aqueous solution. Application of ozone in liquid form in fruit and vegetable processing is often employed for inactivation of pathogen and spoilage micro-organisms [6]. Other studies use ozone in the gas phase such as to change physicochemical [7], ozone-oxidized starches from white and red cocoyam, and white and yellow yam cultivars and evaluated for functional, thermal and molecular properties [8], and oxidation of potato starch using a high concentration of aqueous ozone [9]. However, few studies have investigated the changes of starch properties associated with ozone treatment, particularly when applied in aqueous solution at low concentration. Thus it is necessary to study the oxidation of starch using low concentrations of dissolved ozone and evaluated physicochemical properties of ozone oxidized starch.

2. Material and method

2.1. Material
Cassava cultivar Adira 4 was obtained from Cangkringan, Sleman, Yogyakarta, Indonesia, Oxygen (O₂), N,N-diethyl-p-phenylenediamine (DPD) test kit, HCl solution, NaOH solution, Iodine solution, Acetic acid solution, Hydroxylamine Hydrochloride, and others. Reagents used in this work were analytical grade.

2.2. Preparation of ozone-oxidized starches
Native cassava starch was mixed with a known volume of distilled water to get material:liquor ratio 1:6 and 1:10. Starch slurries were circulated through the ozone generator. The ozone is produced by ozone generator unit using corona-discharge method. Ozone concentration was determined by direct method using N,N-diethyl-p-phenylenediamine (DPD) test kit, the oxidant contained in the sample immediately reacted with the DPD and formed pink color. Oxidant concentration was then determined using color comparator to find out ozone concentration (ppm) [10]. Starch slurries were reacted with dissolved ozone at various concentrations (0.8, 1.4 and 2 ppm) for 5 min. The pump was turned on to circulate the starch slurry and dissolved ozone. After the oxidation process, the oxidized starch was precipitated for 2 h and dried at 50 °C till the constant weight was obtained. Dried oxidized starches were then ground in a mortar until a fine powder was obtained. The powder was then sieved through a sieve of 50 μm.

2.3. Carbonyl content (%)
A starch sample (2 g) was added to 100 mL of distilled water in a 500 mL flask. The suspension was pasted in a boiling water bath for 20 min under constant stirring, cooled to 400°C and adjusted to pH 3.2 with 0.1 N HCl. Hydroxylamine (15 mL) was then added to the mixture. The flask was placed in a 40 °C water bath for 4 h with slow stirring. The excess hydroxylamine was determined by rapidly titrating the reaction mixture to pH 3.2 with standardized 0.1 N HCl. A blank determination with only the hydroxylamine reagent was performed in the same manner. The hydroxylamine reagent was prepared by dissolving 25 g of hydroxylamine hydrochloride in 100 mL of 0.5 N NaOH before the final volume was adjusted to 500 mL with distilled water [11]. The carbonyl group content was calculated as follows: Percentage of carbonyl content = ((blank-sample) mL x acid normality x 0.028 x 100)/sample weight (dry basis) in g

2.4. Carboxyl content (%)
Approximately 2 g of a starch sample were mixed with 25 mL of 0.1 N HCl, and the slurry was stirred occasionally for 30 min with a magnetic stirrer. The slurry was then vacuum-filtered through a 150 mL medium porosity fritted glass funnel and washed with 300 mL of distilled water. The starch cake was then 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 titrated to pH 8.3 with standardized 0.01 N
NaOH. A blank test was performed with unmodified starch [12]. The carboxyl content was expressed as the quantity of carboxyl groups per 100 glucose units (COOH/100 GU). The carbonyl group content was calculated as follows:

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\text{mequiv of acidity/100 g of starch} = \frac{((\text{sample-blank}) \times \text{normality of NaOH} \times 100)}{\text{sample weight (dry basis)}} \text{ in g. Percentage of carboxyl content} = \left(\frac{\text{mequiv of acidity/100 g of starch}}{0.045}\right)
\]

2.5. Amylose content
Amylose content determined by a procedure described in AOAC (2004) [13]

2.6. Swelling and Solubility
A starch sample of 1.0 g was accurately weighed and quantitatively transferred into a clear dried test tube and re-weighed (W₁). The starch was then dispersed in 50 ml of distilled water. The resultant slurry was heated at 95°C for 30 min. The mixture was cooled to 30°C and centrifugated (500 rpm, 20 min). Aliquots (5 ml) of the supernatant were dried to a constant weight at 110 °C. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. Solubility was calculated as g per 100 g of starch on a dry weight basis. The residue obtained from the above experiment (after centrifugation) with the water it retained was quantitatively transferred to the clean dried test tube used earlier and weighed (W₂) [14].

Swelling of starch = W₂ - W₁/weight of starch

2.7. Paste clarity
Paste clarity was evaluated by the transmittance measurement (T%) which was carried out by a procedure described by Craig et al (1989)[15] and was modified by Aplevicz and Demiate (2007) [16]. Samples were prepared by mixing 0.2 g of starch in 20 mL of distilled water in test tubes with screw cap. The tubes were then heated in a boiling water bath for 30 min and stirred individually every 5 min. Samples were then subsequently cooled at room temperature and determined using GENESYS 10S UV-VIS Spectrophotometer (USA) at 625 nm. The percentage of transmittance (%T) was determined at wavelength 650 nm against a water blank.

2.8. Pasting properties
The pasting properties were determined using RVA-4 (Newport Scientific Pvt. Ltd., Australia, with the Thermocline for Windows software, version 3.0). A suspension of 3 g (correct to 14% moisture basis) starch in 25 g of distilled water was analyzed according to a programmed heating, retention and cooling cycle under constant shear. The suspension was held at 50°C for 1 min, then heated to 95°C with rate 6°C min⁻¹. The sample was then kept at 95°C for 5 min, followed by cooling to 50°C with rate 6°C min⁻¹ and finally holding at 50°C for 2 min. at 50 C for 2 min. Peak temperature, peak viscosity, trough viscosity, breakdown viscosity, final setback viscosity and final viscosity values were evaluated with the data analysis software provided (Thermocline for Windows, Newport Scientific, Warriewood, Australia).

3. Result and discussion

3.1. Carboxyl and carboxyl contents
Carboxyl and carboxyl content of native and ozone-oxidized starches are presented in figure 1 and figure 2. There are two main reactions occurred during oxidation. First, starch hydroxyl groups are oxidized to carbonyl groups and then to carboxyl groups. This reaction primarily takes place on the hydroxyl groups at the C-2, C-3, and C-6 positions. Second, oxidation also generates degradation of starch molecules by mainly cleaving amylose and amyllopectin molecules at α-1,4 glucosidic linkages [17]. Therefore, the carboxyl and carboxyl contents and the degree of depolymerization in oxidized starch are an indicator of the degree of oxidation. Carboxyl and carboxyl content increased as dilute the concentration of slurry
and concentration of dissolved ozone increased up to 2.0 ppm and 1.4 ppm for carbonyl and carboxyl respectively. The rise of carboxyl content is probably due to further oxidation of the carbonyl to carboxyl groups whereas reaction the decarboxylation occurs. The condition of the process affects the relative amount of the groups formed during oxidation, so the type of the chemical reagent and its affinity with the starch molecule can interfere with these values [17]. Moreover, it is expected that the more glycosidic bonds cleave, the more number of carbonyl and carboxyl formed. The relationship between the formation of carbonyl and carboxyl groups during starch oxidation is not yet completely understood. A consecutive reaction path in which hydroxyl groups in starch molecules are first oxidized to carbonyl groups and then to carboxyl groups [18]. Carbonyl was a primary functional group produced in the ozone-oxidized starches whereas a minor amount of carboxyl groups was formed. The decrease of carboxyl levels occurred in the use of 2 ppm dissolved ozone is also possible some oxidized starch becomes highly degraded and dissolved in water at the next stage of reaction and washed along with the water [17].

![Figure 1. Carbonyl content of native and ozone-oxidized starches](image1)

The same letter notation indicated no significant difference at the 5% probability level.

![Figure 2. Carboxyl content of native and ozone-oxidized starches](image2)

The same letter notation indicated no significant difference at the 5% probability level; a,b,c: indicates slurry concentration; A,B,C: indicates ozone concentration.
3.2. Amylose content
Ozone-oxidation significantly affected the amylose content of modified starches. Amylose content increased as dissolved ozone concentration increased. This can be related to the depolymerization of the starch molecular chain. The oxidation of the starch granules can also be demonstrated by evidence of the hydrolysis of the glycosidic linkages (the covalent bonds between the α-D-glucopyranosyl units). This hydrolysis results in the depolymerization in parts of the amylose and amylopectin molecules, leading to a formation of smaller size chains ([9]. The amylose content of native and ozone-oxidized starches can be seen in figure 3.

![Figure 3. Amylose content of native and ozone-oxidized starches.](image)

3.3. Swelling and solubility
Swelling power and solubility of native and ozone-oxidized starches are presented in figure 4 and figure 5. 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. This causes an increase in granule swelling and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The highest swelling power and solubility were obtained in ozone-oxidized at material: liquor 1:10 with ozone concentration 1.4 ppm. The swelling power increased with increase of concentration dissolved ozone and dilute slurry concentration. The increase of swelling power may be attributed to the formation of hydrophilic groups (–COOH) during oxidation. Carboxylic groups are also known to be electronegative charged and bulkier than the hydroxyl groups so that it causes an electrostatic repulsion between the molecules, being responsible for expanding their chain conformation, making difficult their intermolecular associations, and increasing their solubility. This leads to starches with higher paste clarity and stability ([19]. The rise of starch solubility after oxidation process can also be related to the depolymerization and weakening structure of starch granules.
Figure 4. Swelling power of native and ozone-oxidized starches. The same letter notation indicated no significant difference at the 5% probability.

Figure 5. The solubility of native and ozone-oxidized starches. The same letter notation indicated no significant difference at the 5% probability level. a,b,c: indicates slurry concentration; A,B,C: indicates ozone concentration.

3.4. Paste clarity
Paste clarity of native and ozone-oxidized starches are presented in figure 6. The transmittance of ozone-oxidized starches were greater than native, as well as the storage time which were more stable than native. Mechanism of gel formation and retrogradation are caused by the formation of hydrogen bonds between hydroxyl groups with other chains within the starch granule. Increase of clarity is probably because of the formation of carboxyl and carbonyl groups which can obstruct hydrogen bonds within the granule. In addition, the presence of carboxyl groups which have anionic charge will cause electrostatic repulsion among starch molecules, so that the merger of retrogradation will be blocked [20-23].
3.5. Paste viscosity

Oxidation reactions are always accompanied by hydrolysis of glycosidic bonds within the starch granule. It will lead to the depolymerization resulting in a decrease in molecular weight. Therefore, oxidized starch showed low viscosity [20-23]. The results showed that peak viscosity decreased as ozone concentration increased. The replacement of part of the hydroxyl groups by carbonyl and carboxyl groups and the partial cleavage of the glycosidic bonds. The replacement of the hydroxyl groups had as a consequence the decrease of the hydrogen bonds between the molecules of the modified starches. As these bonds are mainly responsible for maintaining the native starch granules integrity, this integrity is reduced. The carbonyl and carboxyl groups formation can also be suggested as responsible for the granular weakening [18]. Ozone-oxidized starches had lower viscosity than native (8702 cP). The lowest peak viscosity (6043 cP) was obtained in ozone-oxidized starch at material: liquor 1:10 with ozone concentration 1.4 ppm, as shown in figure 7.

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**Figure 6.** Paste clarity of native and ozone-oxidized starches.

**Figure 7.** Amilograph curve of native and ozone-oxidized starches.
4. Conclusion
Ozone-oxidation process using dissolved ozone successfully oxidized cassava starch. Ozone concentration and concentration of slurry significantly affected the physicochemical properties of modified cassava starches resulting in higher amylose content, swelling power, solubility, paste clarity and lower peak viscosity than native. The ozone-oxidation had a different mechanism of oxidation from the common oxidation process by which oxidation with ozone produced more carbonyl groups than carboxyl groups.

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