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Alteration of cassava starch properties after ozone-oxidation treatment in different time

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Abstract

Starch is an essential source of raw materials for food and other industries. One source of starch which is currently abundant and easily obtained is cassava. Ozone is a reliable and safe modifying agent to use. Ozone is non-toxic, harmless to humans and the environment, and it quickly decomposes into oxygen and leaves no waste. The effect ozone-oxidation on cassava starch at a different time (15 min, 30 min, 45 min, and 60 min at 30^{O}C) was investigated for FTIR spectroscopy, pH, X-ray diffraction, WBC and OBC, and morphology of granules starch.

The result showed that FTIR spectra of ozone-oxidized cassava starches did not show any difference compared to native starch. However, the pH and OBC showed decreased with increasing oxidation time and decreased from 7.24-4.09, and 127-99%, respectively. Moreover, the WBC and relative crystallinity (RC) showed risen from 71-97% and 29.90-37.58%, respectively. The morphology of native and ozone-oxidized cassava starch granules showed different from each other.

Keywords: cassava starch, ozone, oxidation time, alteration properties

1. Introduction

Starch is an essential source of raw materials for food and other industries. One source of starch which is currently abundant and easily obtained is cassava (*Manihot esculenta* Crantz). Starch plays an essential role in the texture of food, such as gel-forming, binder, stabilizing, thickening, and bulking agents [1]. Natural starch has some limitations in functional aspects at the industry level and needs to be modified [2]. These limitations include a less clear paste, low thermal stability, easy to retrograde, and low shear resistance [3].

Chemical modification can change starch properties by presenting new functional groups in starch molecules. Starch oxidation is a chemical modification in which the hydroxyl groups of starch molecules are converted to carbonyl groups and subsequently converted to carboxyl groups [4, 5]. Oxidized starch has high thermal stability, low viscosity, low retrogradation, transparent paste, film-forming, and binding properties [3]. There are many chemical reagents used for starch oxidation, such as hydrogen peroxide, sodium hypochlorite, sodium bromate, ammonium persulfate, sodium permanganate, potassium permanganate, and ozone [6]. Among chemical reagents for oxidation, ozone is a safe modifying agent to use. Ozone is non-toxic, harmless to humans and the environment because ozone quickly decomposes into oxygen and leaves no waste. Another advantage of ozone is a realible oxidizing agent because it has a free oxygen atom [7]. As an oxidizing agent, ozone has been used extensively in water treatment, food processing, and environmental concerns [8]. Oxidized starches have been applied in butter and breading for coating in various food products, as binders in confectionary and film formers, in dairy texturizers, and paper industry [9].

At present, although starch oxidation research has been carried out, the information on cassava starch oxidation using ozone is still limited. Knowledge of the characteristics of oxidized cassava starch is essential for determining applications in the food and other industries. Therefore, this study aims to evaluate the effect of ozone-oxidation for 15 min - 60 min at 30^{O}C on cassava starch properties, such as the spectrum profiles of Fourier transform infrared spectroscopy (FTIR), pH, the X-ray diffraction patterns, water binding capacity (WBC), oil binding capacity (OBC) and the surface morphology of granules.

2. Materials and Methods

2.1 Materials

Cassava starch from gajah varieties was obtained from small industry in Kutai Kartanegara, Indonesia.

2.2 Chemicals and reagents

Oxygen, DPD test and Chemical reagents were Pro Analysis grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3 Preparation of Ozonated Gajah cassava starch

The method refers to Castanha *et al.* [10] with small modifications. The 10% starch suspension flow with 2 ppm dissolved ozone from ozone generator and stiring during oxidation for 0, 15, 30, 45 and 60 min at 30^{O}C. The slurry and liquid supernatant were separated. The slurry was dried at 55^{O}C for 12 h using a cabinet dryer and milled and sifted with a 100-mesh sieve. Dried oxidized starch was storaged in a container for further analysis.

2.4 Fourier transforms infrared (FTIR) spectroscopy

FT-IR spectra of different oxidized starches were analysed using a Fourier transform infrared spectrometer (Nicole Nexus 670, USA) at The Integrated Research and Testing Laboratory (LPPT) UGM, with 4 cm^{-1} of resolution. The transmittance spectra were measured in a range of wavenumber from 400 to 4000 cm^{-1}.

2.5 pH

The pH of the ozone-oxidized cassava starch samples was measured based on the method of Adeleke and Odedeji[11] using a pH meter (OHAUS, starter 5000). The pH meter was calibrated before used, with buffer solutions of pH 4.0 and 6.86. Each starch sample (10%) was mixed and homogenized with distilled water and the suspensions were measured and the value of pH values were recorded.

2.6 Water and oil binding capacity

The water binding capacity (WBC) of starches was determined using the method of Yousif *et al.* [12], Adeleke and Odedeji [11]. The starch suspension was prepared by dissolving 1 g of starch in 15 ml distilled water, agitated for 2 mins and centrifuged at 1250 × g for 20 mins. The supernatant was decanted and wet ozone-oxidized cassava starch was weighed. The amount of water (%) absorbed by the sample was reported as water binding capacity. For oil binding capacity (OBC) measurement, 1 g sample of native and ozone-oxidized cassava starch was weigh in a 25 ml centrifuge tube, 10 ml refined palm oil was added to the sample. The tube was agitated for 2 min using a vortex mixer and then centrifuged at 1250g for 20 min. The amount of oil (%) absorbed by the sample was reported as oil binding capacity.

2.7 Starch X-ray diffraction pattern

The samples were subjected to an X-ray diffractometer (Rigaku Miniflex 600 RXD, Tokyo, Japan) with copper radiation at an angle 2 theta ranging from 3 to 40 ^{O}C, using the working conditions: scan rate of 0.5^{O} min^{-1}, 40 kV and 15 mA. The relative crystallinity (RC) of the starch granules was samples was counted using the Origin pro software version 2017 (OriginLab corp., Northampton, MA 01060, USA).

2.8 Morphology of the starch granules

The method refers to Castanha *et al.* [10] with small modifications. The morphology of the native and ozone-oxidized cassava starch granules were determined using a scanning electron microscope (JSM-6510 Jeol). A very few quantities of the native and ozone-oxidized cassava starch were spread on to the surface of the plate's sample. All the starch samples were coated with gold and analyzed in the scanning electron microscope under an acceleration voltage of 15 kV and observed with magnifying of 1000x and 5000x.

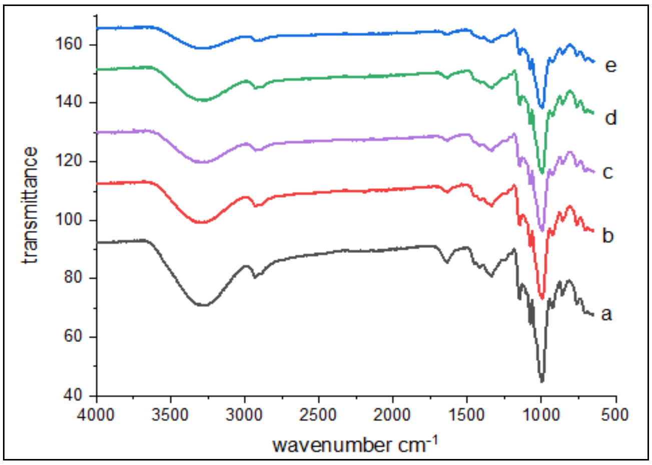
2.9 Statistical analysis

The research data were analyzed using one-way ANOVA with SPSS Version 25. If the F value was greater than the F table value, Duncan's Multiple Range Test at $\alpha = 5\%$ was used to calculate if there was a difference between treatments.

3. Results and Discussion

FTIR spectroscopy analysis

Native cassava starch and ozone-oxidized cassava starches shown more than ten peaks in the spectra of 4000–500 cm^{-1}(Fig.1). The peaks of wideband at 3286 cm^{-1} can be represented to O–H stretching vibration, and the peak at 2927 cm^{-1} can be ascribed to C–H bond stretching [13]. The transmittance of starch at 1635 and 1414 cm^{-1} were confirmed the presence of C–O–C stretching and C–H bending, respectively [14]. The peak at 1148 and 1076 cm^{-1} are both ascribed to the coupling of C–O, C–C and O–H bond stretching, bending and asymmetric stretching of the C–O–C glycosidic bridge [15]. The peak at 996 cm^{-1} was attributed to the vibration of C–O–H deformation [16], and transmittance at 925 and 860 cm^{-1} were ascribed to both for C–H bending [13].



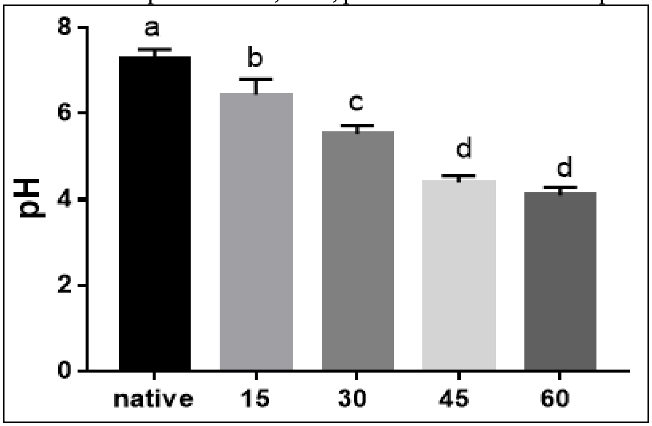
a: native cassava starch, b, c, d, and e: ozone-oxidized cassava starch at 15, 30, 45 and 60 minutes, respectively.

Fig 1: FTIR spectra of native and ozone-oxidized cassava starches

Oxidation of starch produced the changed of hydroxyl groups into carbonyl and carboxyl groups, and this conversion in these groups was checked using FTIR spectroscopy. However, the FTIR spectra of ozone-oxidized cassava starches at 15, 30, 45 and 60 minutes did not show any difference compared to native starch. The result may be caused to the formation of smaller carbonyl and carboxyl groups during ozone-oxidation. Similar results for no change in FTIR spectra of oxidized starches at different modification levels have been reported for barley starch [17]. However, Zhang *et al.* [18] mentioned there were additional peaks at 1735.6 cm^{-1} in oxidized starch from corn starch, pea starch, and sweet potato starch due to high oxidation degree (> 40.5%) gained in their research.

pH

Fig 2. showed the pH of ozone-oxidized cassava starch lower compared to the native cassava starch. The longer the oxidation time, the pH sample more decreased significantly. It decreased by about 40% compared to the pH initial of the sample, from 7.24 to 4.09. According to Castanha *et al.* [10], starch molecules rich in hydroxyl groups and oxidation substituted for hydroxyl groups into carbonyl groups and subsequently transform into carboxyl groups. The carboxyl group is the final product of the oxidation process. Carboxyl groups exist in the form of carboxylic acids. The longer the oxidation time, the higher the carboxyl groups formed. It caused the acidity of the sample increases, and the pH of the sample decreases. Ozonation of potato starch for 1 hour can reduce the pH from 6.75 to 4.20 [10]. Similar results reported by Catal and İbanoğlu [19] for 1 hour ozonation significantly decreased the pH of wheat, corn, potato and rice flour samples.

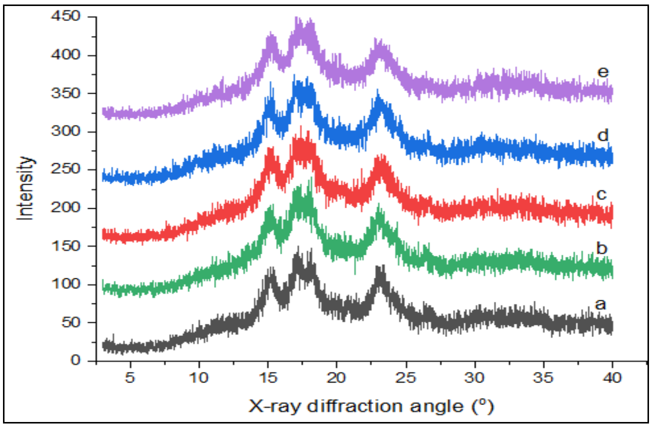


¹Values are given as the Means±SD of the triplicate measurements. Histograms followed by the same superscript letter show not significantly different from the DMRT at α = 5%.

Fig 2: pH of native and ozone-oxidized cassava starches.

Starch X-ray diffraction pattern

Fig. 3 shows the diffraction peaks of native and ozone-oxidized cassava starch were observed at diffraction peaks 2-theta of 15°,17, 18° and 23°. The relative crystallinity (RC) of ozone-oxidized cassava starch increases with increasing oxidation time. The relative crystallinity of ozone-oxidized cassava starch was higher than the native cassava starch. The relative crystallinity of native and ozone-oxidized cassava starches with oxidation times of 15 min, 30 min, 45 min, and 60 min was 29.90%, 32.47%, 34.85%, 36.28%, and 37.58%, respectively. The highest RC was ozone-oxidized cassava starch at 60 minute oxidation. An increase in RC, possibly due to increasing the acidity levels as confirmed in graph 2. resulting in acid hydrolysis. Junior *et al.* [20], reported ozone could use as an alternative to the fermentation process. Zhao *et al.* [21], mentioned an increasing RC in fermented wheat starch from 37.15% to 42.27% compared with native starch. Lu *et al.* [22], also reported an increase in relative crystallinity rice flour from 33.81% to 39.37% after fermentation.



a: native cassava starch, b, c, d and e: ozone-oxidized cassava starch at 15, 30, 45 and 60 minutes

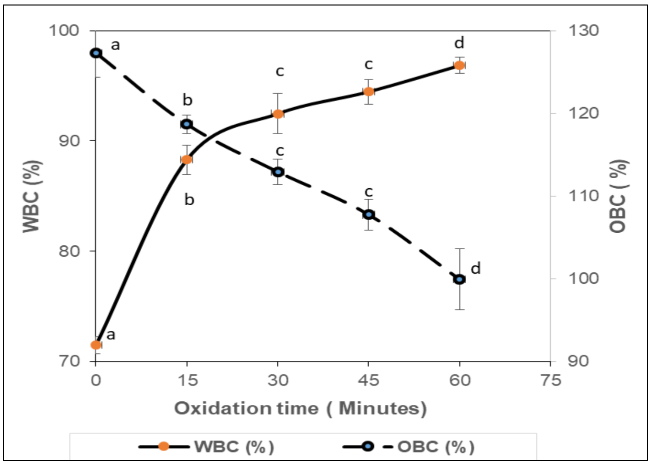
Fig 3: XRD spectra of native and ozone-oxidized cassava starches

Some hypotheses proposed for the increased relative crystallinity in the initial stages of acid hydrolysis. Firstly, Reordering of the newly released chain due to the cleavage of several amylose chains through the amorphous regions may allow ends into a more crystalline structure [23, 24]. Secondly, Partial filling of water channels in the crystallite cavities with double helices so reordering of the crystalline structure [25]. Thirdly, Retrogradation of free amylose, which hydrolized become double helices, and rearranges into crystalline regions [26, 27]. In the starch granules, Hydrated amylopectin molecules could behave as liquid crystal polymers. Decoupling of individual double helices from the amylopectin backbone by acid hydrolysis could remove spatial constraints and allow double helices to alignment into more crystalline structures [28, 29, 30].

Water binding capacity (WBC) and Oil binding capacity (OBC)

Fig.4 showed the WBC of ozone-oxidized cassava starch increases significantly with an increase in oxidation time, and contrast to OBC. The hydrophilicity rose while the hydrophobicity fell after oxidation. According to Castanha *et al.* [10] the increase in WBC was a result of the higher introduction of carbonyl and carboxyl groups to the starch molecules, which facilitated an enhanced water-binding in oxidized starch. Adebowale *et al.* [31] found that WBC of the oxidized Sword Bean starch increase 22.73% compare to the native starch. The WBC of oxidized starches depends on the availability of water binding sites, which is determined by the type and intensity of oxidation. The oxidation treatments typically cause weakening of hydrogen bonds and favoring the penetration of water into the granules [31, 32].

The OBC ozone-oxidized starch singnificantly decreased compared to native cassava starch. The values expressed as percentage varied from 127% observed for native cassava starch and 118- 99% found observed for oxidized samples. Similar results have reported on the oxidation of Acha starch. The oxidized acha starch have OBC lower than OBC from native Acha starch [33]. Sathe and Salunkhe [34] mentioned the increase in starch crystallinity restricted access of oil into the granule of the starch. The oxidation starch is thought to occur in the amorphous region of the starch molecules leading to increase in starch crystallinity. Mudgil *et al.* [35], mentioned guar gum with high WBC could use as a thickener, controlling syneresis, prevention of fat migration during storage, and controlling viscosity and rheology in meat processed product. There is an opportunity to use ozone-oxidized cassava starch with high WBC resemble the character of guar gum in food products.

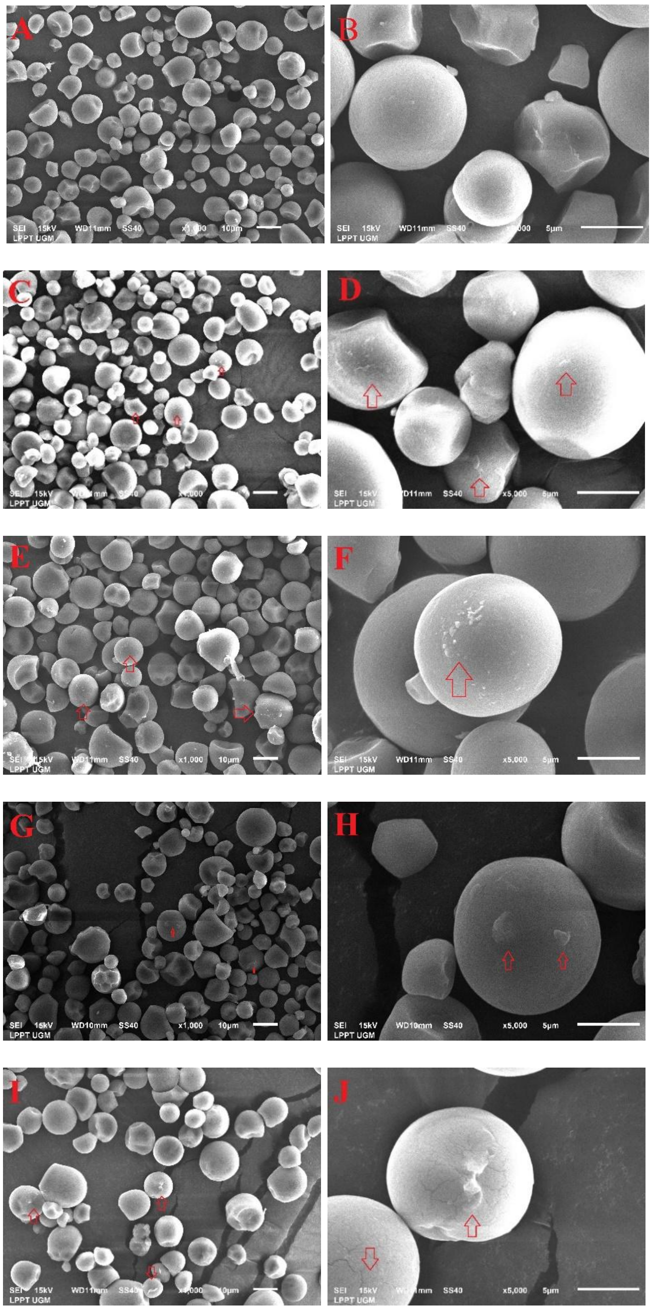


¹Values are given as the Means±SD of the triplicate measurements. Curve followed by the same superscript letter show not significantly different from the DMRT test at α = 5%.

Fig 4: WBC and OBC of native and ozone-oxidized cassava starches

Morphology of granules starch

Morphology of the native and ozone-oxidized cassava starch granules was shown in Fig. 5. Scanning electron micrographs were used to investigate whether the ozone oxidation at a different time affected on the surface morphology of the starch granules. The native cassava starch showed the smooth surface and round shape without evidence of gaps or pores (Fig. 2a and b). It was appropriate, as is usually observed in the native cassava starch granules [36]. Oxidized cassava starch granules present a rough surface (indicated by red arrows in Figures 2c, d, e, f, g, h and i).



magnifying 1000X magnifying 5000X

A, B = Native cassava strach, C, D =15 minutes oxidation, E,F = 30 minutes oxidation, G, H = 45 minutes oxidation, and I, J = 60 minutes.

Fig 5: Morfology of native and ozone-oxidized cassava starch granules

The surface damage of the oxidized starch granules increasing with increasing oxidation time. It was shown in Figure 2 d, f, h, and J. The surfaces defect of the granules starch was demonstrated clearly with a 5000-fold magnification. Fig. 2J showed the granule surface with the most significant roughness, it was slightly cracked and peeled off and probably due to the longest oxidation time, by 60 minutes. Similar results were reported on the oxidation of cassava starch using peroxide and hypochlorite [37]. Catal and Ibanoglu [19] mentioned ozone-oxidation on potato and corn. All the native starch samples showed surfaces smooth and regular, while after ozonation, the surface granule became rough and fibrous, particularly in the potato starch. Vanier *et al.* [9], mentioned oxidization using 1.5% level of active chlorine, the starches granules of common bean showed imperfections on their external structures and a coarser surface than that of native starch granules.

The data have reported above, supports the assumption that the ozone-oxidized starch in this study has the potential for food ingredients with specific purposes.

4. Conclusions

Ozone oxidation in cassava starch is a chemical modification. The results showed a significant influence on pH, X-ray diffraction pattern, water binding capacity, oil binding capacity, and surface morphology of cassava starch granules. Whereas FTIR spectroscopy of ozone oxidized cassava starch has a pattern similar to the native cassava starch. Ozone oxidation for 60 minutes has the highest effect on cassava starch properties. Oxidized starch in this study has the potential for ingredients in food products with specific purposes.

5. Acknowledgment

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