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Effects of photooxidation exposure time on the modification of cassava starch: a comprehensive study on chemical, functional, structural, and morphological properties

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Abstract

Photooxidation is one of the green technologies that can be used to modify starch. This process produces oxidized starch which induces alterations in the starch structure and functional properties. Photooxidation of starch can be influenced by hydrogen peroxide concentration, exposure time, slurry concentration, ultraviolet (UV) intensity, addition of lactic acid, and temperature. This study aims to evaluate the effect of photooxidation exposure time on the chemical, functional, structural, and morphological properties of cassava starch. Cassava starch was modified using a UV catalysator reactor and treated with photooxidation using a combination of hydrogen peroxide and UV irradiation for 15, 30, and 45 min. The result showed that the longer exposure time produced starch with higher amylose, carboxyl contents, solubility, water absorption capacity, oil absorption capacity, and color L*. Moreover, the longer time of the photooxidation process caused a decrease in the pH value, swelling power, and pasting properties. Photooxidation decreased the relative crystallinity values and damaged the granule morphology of the modified cassava starch. These results showed that photooxidation successfully modified cassava starch.

Keywords: cassava starch; photooxidation; starch properties.

Practical application: Optimizing photooxidation time enhances cassava starch properties for diverse industrial applications.

1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a staple crop cultivated in both tropical and subtropical regions, including Indonesia. Cassava serves as a starch source, containing approximately 80% starch on a dry weight basis (Mejía-Agüero et al., 2012). Native cassava starch exhibits weaknesses, such as low solubility, freeze-thaw instability, shear and thermal resistance, poor enzymatic hydrolysis, high syneresis, a propensity toward retrogradation, and high digestibility (Gunorubon & Kekpugile, 2012). Therefore, to overcome the limitations of natural cassava starch and broaden its functional properties and applications, modifications to the starch, whether physical, chemical, or enzymatic, are essential.

Ultraviolet (UV) irradiation represents a nonthermal and environmentally friendly technology for starch modification. The combination of UV irradiation with lactic acid fermentation in cassava starch has been reported to induce physicochemical changes that result in lower viscosity and increased stability against retrogradation. These modified starch characteristics make it suitable for application in cheese bread (Santos et al., 2021). This indicates that UV irradiation brings about alterations in the structure of starch, impacting its functional properties.

The utilization of UV irradiation in starch modification coupled with hydrogen peroxide application (referred to as photooxidation) yields oxidized starch (Ekafitri et al., 2021; Tethool et al., 2012b). Several studies reveal that oxidation using hydrogen peroxide results in a degree of oxidation of less than 2.78% (Zhang et al., 2012). To enhance this degree of oxidation, UV irradiation can be employed as it does not leave residues, unlike metal catalysts such as Cu and Fe. The combined use of hydrogen peroxide and UV irradiation leads to an increased degree of oxidation, characterized by a greater rise in the number of carbonyl and carboxyl compared to when hydrogen peroxide and UV are used individually. The modification through hydrogen peroxide and UV irradiation (photooxidation) induces alterations in the starch structure and functional properties, expediting the starch modification process for enhanced suitability in industrial applications (Hornung et al., 2016).

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The investigation into cassava starch modification utilizing UV irradiation has been explored in previous studies, including combinations with lactic acid and UV (Dias et al., 2011; Santos et al., 2021) and esterification combined with UV (Sumardiono et al., 2019) and a composite approach involving lactic acid, hydrogen peroxide, and UV (Muflihati et al., 2019). However, the application of a combined modification strategy involving hydrogen peroxide and UV (photooxidation) for cassava starch remains relatively unexplored. The effect of the photooxidation exposure time on cassava starch modification has never been explored before. Therefore, this study aims to evaluate the effect of the photooxidation exposure time on the chemical, functional, structural, and morphological aspects of cassava starch.

2 MATERIALS AND METHODS

2.1 Materials

The main material used in this research was commercial cassava starch (*Sagu Pak Tani*) obtained from the Subang market, West Java, Indonesia. In addition, the chemicals used include NaOH, HCl, hydroxylamine chloride, indicators PP, KI, H_2SO_4, Na-thiosulfate, Luff-Schoorl solution, acetic acid, ethanol, iodine solution, and other pro-analysis chemicals.

2.2 Preparation of modified starch by photooxidation

Starch modification by photooxidation was carried out using a UV catalysator (Figure 1) referred to Ekafitri et al. (2021). Starch slurry with a starch: water ratio of 1:6 (w/v) was put into a holding tank, and then hydrogen peroxide was added at a concentration of 3%. Next, the slurry was pumped and flowed into a UV irradiation tube. In the irradiation tube, the UV lamp was turned on to provide irradiation to the flowing flour slurry according to treatment times of 15, 30, and 45 min). Mechanical stirring was implemented during the oxidation process to prevent starch precipitation. Following the oxidation process, the starch slurry was removed, washed, and deposited for 3 h. The starch was subsequently separated from the liquid and dried using a dryer cabinet at 50°C for 12 h. The dried starch was then ground using a blender (Philips HR2115, Indonesia) and stored at 25°C until for analysis.

2.3 Determination of physicochemical properties

Physicochemical properties of samples included carboxyl content (Demiate et al., 2000), amylose (Perez & Juliano, 1978), swelling power (SP) and solubility (Tester & Morrison, 1990), water absorption capacity (WAC) (Subroto et al., 2019), oil absorption capacity (OAC) (Huang et al., 2019), pH (Benesi et al., 2004), and color (L*, a*, and b* values) using a colorimeter (Konica Minolta CM 700d, Japan); Fourier-transform infrared (FTIR) measurements were carried out using a FTIR spectrometer ALPHA II (Bruker instrument, Billerica, MA-USA); pasting/amylography properties were measured using a Rapid Visco Analyzer (RVA) (RVA-Tec Master Newport Scientific, Australia) referred to Kaushal et al. (2012); the crystallinity pattern was measured using a X-ray diffractometer (Bruker D8 Advance ECO, Germany) referred to Klein et al. (2013); and the morphology and surface of modified flour granules were examined using scanning electron microscopes (SEMs)

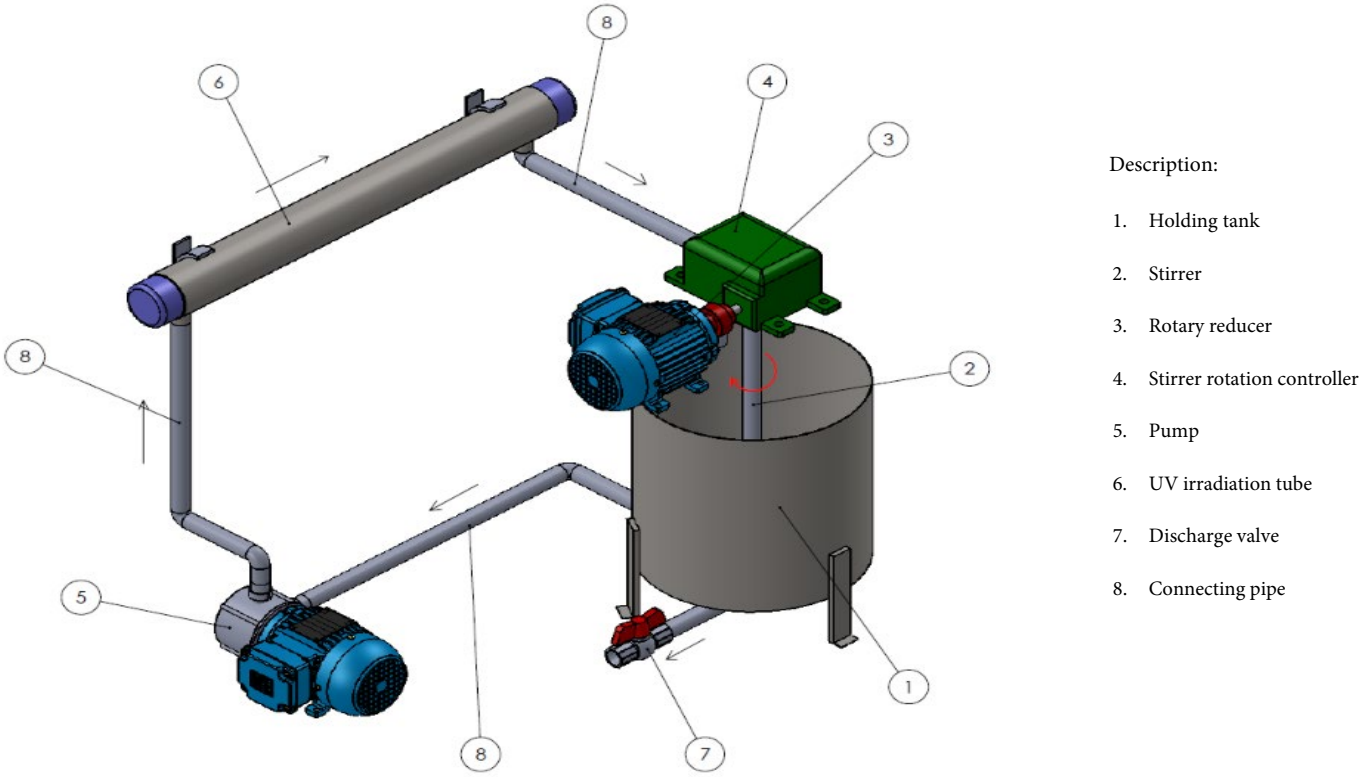

Figure 1. UV catalysator reactor.

(SEM Hitachi SU3500, Japan) referred to Huang et al. (2016). Native starch/treatment samples were previously coated with a layer of Au 20 mA for 60 s with a voltage of 3 kV. The prepared samples were placed on a silver plate and then examined and photographed using SEM at magnification 4,000× and 9,000×.

2.4 Statistical analysis

The SPSS version software (SPSS, Inc., USA) was utilized to perform a one-way analysis of variance with the Duncan test, and statistical significance was considered at $p < 0.05$. All of the data were subjected to triple measurement and performed as mean ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 Physicochemical properties

The carboxyl content increased with increasing exposure time. Photooxidized cassava starch has carboxyl content (0.0469%–0.0875%) higher than native cassava starch (0.0469%) (Table 1). There was a 32.77% rise in carboxyl content from 15 to 45 min (Table 1). Extending the exposure time to UV photooxidation has been established to enhance the carboxyl content in cassava starch (Muflihati et al., 2019). This phenomenon is attributed to the oxidation process, wherein hydroxyl groups within starch molecules undergo oxidation, transforming into carbonyl groups and subsequently evolving into carboxyl groups (Zavareze et al., 2012). Notably, the conversion from carbonyl to carboxyl is observed to occur at a faster rate than the transformation from hydroxyl to carbonyl (Sangseethong et al., 2010). As exposure time increases, a greater number of hydroxyl groups undergo oxidation, leading to a significant augmentation in the number of carboxyl groups (Kuakpetoon & Wang, 2006), indicative of the intensification of the oxidation process.

The results of the amylose content test presented in Table 1 demonstrate a significant increase in conjunction with the duration of photooxidation exposure. The highest amylose content, reaching 54.64%, was observed following a 30 min treatment, suggesting that extended UV irradiation for 30 min creates optimal conditions for augmenting amylose levels in cassava starch. This phenomenon can be attributed to the prolonged reaction time, leading to the depolymerization of amylopectin chains into amylose with shorter molecular chains and a higher quantity, as well as the formation of intermolecular cross-links (Kuakpetoon & Wang, 2008).

The depolymerization of amylopectin results in the breaking of branch chains within the amylopectin molecule, causing a transformation of the molecular structure from amylopectin to the simpler, more linear structure of amylose (Kuakpetoon & Wang, 2006). Starch oxidation induces degradation and molecular breakdown of both amylose and amylopectin at α-1,4-glycosidic linkages (Wang & Wang, 2003). However, after 45 min, a decrease in amylose content was observed, possibly attributed to the depolymerization of amylose chains into polymers with shorter chains, such as oligosaccharides.

Table 1 reveals a significant increase in solubility in photooxidized cassava starch (5.20–8.20%) compared to native cassava starch (5.05%) ($p < 0.05$). Prolonged exposure time further demonstrates an augmentation in solubility for photooxidized cassava starch. This enhanced solubility is attributed to the weakening of the internal granule structure of starch and the depolymerization of amylose due to hydrogen peroxide and UV oxidation (Sandhu et al., 2008). The breakdown of amylose segments contributes to the observed increase in solubility (Halal et al., 2015). SP is the starch's ability to undergo hydration under specific conditions (Vanier et al., 2017) determined by measuring the weight of swollen starch granules and the absorbed and retained water within the starch granules (Falade & Okafor, 2015). The SP of native cassava starch is 8.39 g/g, significantly higher than that of photooxidized cassava starch (4.66–5.56 g/g) ($p < 0.05$). Prolonged exposure time leads to a decrease in SP values. This trend aligns with the findings in the oxidation of barley starch (Halal et al., 2015), pinto bean starch (Vanier et al., 2012), and taro flour (Ekafitri et al., 2021). The reduction in SP in oxidized starch is attributed to the depolymerization of amylopectin chains and the formation of a sponge-like structure within the granules, capable of imbibing water during heating but unable to retain absorbed water during the centrifugation process.

The pH value of photooxidized starch ranged from 3.39 to 5.56, which was lower compared to native cassava starch (pH 3.58), and the pH exhibited a significant decrease with prolonged exposure time ($p < 0.05$). This decline in pH is attributed to the formation of acidic groups such as carbonyl, carboxyl, and peroxide due to oxidation by hydrogen peroxide and UV

Table 1. Physicochemical of native and photooxidized cassava starch.

Samples	Parameters*									
	Carboxyl content (%GU)	Amylose content (%)	Solubility (%)	SP (g/g)	pH	WAC (%)	OAC (%)	L*	a*	b*
Native starch	0.0469 ± 0.00^{a}	41.17 ± 0.48^{a}	5.05 ± 0.05^{a}	8.39 ± 0.03^{d}	5.58 ± 0.00^{d}	188.85 ± 2.34^{a}	188.94 ± 0.80^{a}	93.13 ± 0.01^{a}	-0.38 ± 0.01^{b}	2.79 ± 0.01^{b}
Photooxidized starch at exposure times of 15 min	0.0659 ± 0.00^{ab}	48.23 ± 0.15^{b}	5.20 ± 0.11^{a}	5.56 ± 0.24^{c}	5.56 ± 0.00^{c}	195.32 ± 3.36^{b}	197.81 ± 3.61^{b}	94.81 ± 0.37^{b}	-0.51 ± 0.05^{a}	1.95 ± 0.13^{a}
Photooxidized starch at exposure times of 30 min	0.0666 ± 0.00^{ab}	54.64 ± 0.06^{c}	6.47 ± 0.06^{b}	5.19 ± 0.15^{b}	5.25 ± 0.01^{b}	202.34 ± 1.77^{c}	221.33 ± 0.14^{c}	95.14 ± 0.15^{b}	-0.50 ± 0.03^{a}	1.90 ± 0.10^{a}
Photooxidized starch at exposure times of 45 min	0.0875 ± 0.00^{ac}	52.08 ± 0.39^{d}	8.20 ± 0.17^{c}	4.66 ± 0.11^{a}	3.93 ± 0.02^{a}	207.98 ± 1.60^{d}	227.83 ± 3.06^{d}	95.01 ± 0.19^{b}	-0.48 ± 0.01^{a}	2.02 ± 0.02^{a}

*The same superscript in the same column shows that the samples were not significantly different at 5% significance.

exposure, accompanied by an increase in carbonyl and carboxyl content. Studies by Banura et al. (2018), Carvalho et al. (2021), and Guo et al. (2022) have noted that oxidation induced by plasma (containing UV) leads to a decrease in pH in banana, yam, and potato starch.

The WAC and OAC of photooxidized cassava starch exhibit a similar trend, increasing with prolonged exposure time to photooxidation, with values significantly different from native starch ($p < 0.05$) (Table 1). Native cassava starch has WAC and OAC values of 188.85 and 188.94%, respectively. In contrast, modified cassava starch displays WAC and OAC values ranging from 195.32 to 207.98% and 197.81 to 227.83%, respectively. The findings of this study align with the research conducted by Okekunle et al. (2020), suggesting that oxidation can enhance the WAC and OAC values of lima bean starch. The greater WAC value in modified starch compared to native starch is attributed to the oxidation process, which breaks down starch molecules into simpler forms capable of retaining water and increasing water absorption capacity (Banura et al., 2018). Additionally, the oxidation process leads to the formation of a porous surface, creating conditions favorable for more efficient water and oil absorption. The pores and crevices on the starch surface facilitate the entry of water and oil molecules, contributing to the increased WAC and OAC of cassava starch (Chelule et al., 2010).

The photooxidation process induces an increase in the L* value and a decrease in the a* and b* values of cassava starch. The exposure time does not result in significant changes in the L*, a*, and b* values ($p > 0.05$) but differs significantly from native starch ($p < 0.05$). The native cassava starch exhibits L*, a*, and b* values of 93.13, -0.38, and 2.79, respectively. In contrast, the L*, a*, and b* values of photooxidized cassava starch are 94.81–95.01, -0.48 to -0.51, and 1.90-2.02, respectively. This aligns with research on oxidation-UV irradiation in sago starch (Tethool et al., 2012a).

The increase in the L* value is attributed to the fact that in the oxidation-UV irradiation reaction, some carotenoid pigments and proteins oxidize before the glucose units, resulting in the partial loss of these compounds and ultimately producing whiter starch (Vanier et al., 2012). In the oxidation reaction, as indicated by Sánchez-Rivera et al. (2005), some pigments and proteins oxidize before the glucose units, leading to the partial loss of these compounds and causing a reduction in the yellow color intensity in the flour (b* value). The same is presumed to occur in the reduction of the red color intensity (a* value) in the flour.

3.2 Fourier-transform IR

The FTIR pattern showing the functional groups in native cassava starch and modification by photooxidation can be seen in Figure 2. A peak at a wave number of 1,740 cm^{-1} in the modified cassava starch, while this peak is not visible in the native cassava starch. This peak indicates the presence of a C=O carbonyl group (Nandiyanto et al., 2019). This means that photooxidation has successfully changed the starch hydroxyl groups into carbonyl and carboxyl groups, which shows that the oxidation process has occurred. The results of this study are similar to starch oxidation using ozone, where oxidized starch shows a peak at around 1,700 cm^{-1} which is the carbonyl group (Satmalawati et al., 2020). The peak at wave number 3,275 cm^{-1} has a lower intensity in oxidized cassava starch compared to native cassava starch. The peak at wave numbers 3,300–2,500 cm^{-1} indicates the presence of hydroxyl O-H stretching bonds (Satmalawati et al., 2020). The lower intensity shows that there are fewer hydroxyl groups due to oxidization to carbonyl and carboxyl, similar to research results (Han, 2016).

3.3 Pasting properties

Photooxidized cassava starch exhibited lower peak viscosity (PV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV) compared with native cassava starch ($P < 0.05$) (Table 2). In general, the decline in pasting properties is caused by the oxidation process through hydrogen peroxide and UV, which leads to the weakening of the molecular and granular structure of starches. This weakening happens due to an increase in the carbonyl and carboxyl group contents, as well as the depolymerization of glucose polymers (Vanier et al., 2017). Previous investigations have observed similar results that the oxidative modification caused a strong decrease in the FV of cassava starch (Hornung et al., 2016, 2018). The reduction in SV is attributed to the existence of carbonyl and carboxyl groups in larger starch molecules compared to hydroxyl groups. This leads to the separation of amylose chains, hence inhibiting the retrograde process.

The decline in PV observed in oxidized starches may be attributed to the partial cleavage of glycosidic bonds during hydrogen peroxide treatment, resulting in a reduction in the molecular weight of starch molecules and subsequently causing a decrease in viscosity (Chan et al., 2009; Vanier et al., 2012). However, beyond the 30-min mark, there is an increase in PV, possibly induced by carbonyl and carboxyl groups. These groups stimulate swelling in the granules and lead to the formation of hemiacetal or hemiketal cross-links through the oxidation process, occurring predominantly along the amylopectin molecule and to a lesser extent between the amylopectin and amylose molecules (Ekafitri et al., 2021; Vanier et al., 2012).

BV of photooxidized cassava starch tended to decrease at 15 and 30 min exposure time but then increased at 45 min exposure time (Table 2). The decrease in the BV of cassava starches occurred due to the incorporation of novel substituent groups into the oxidized starches (Sandhu et al., 2008). Previous research discovered lower BV, improved heat stability, and shear resistance of starch (Okekunle et al., 2020).

The SV and FV of photooxidized cassava starch exhibit a similar trend, increasing during 15 min and 30 min of exposure and decreasing at 45 min of exposure (Table 2). The observed rise in SV is attributed to starch retrogradation, involving the association of nearby amylose molecular chains through intermolecular hydrogen bonding. Meanwhile, the decline in retrogradation tendency is associated with the presence of carbonyl and carboxyl groups on starch molecules that are bulkier compared to hydroxyl groups, leading to a tendency to keep amylose chains separated and hinder retrogradation (Liu et al., 2014; Martínez-Bustos et al., 2007; Tavares et al., 2010). Food products benefit from starches with low setback values, indicative of a reduced inclination for retrogradation.

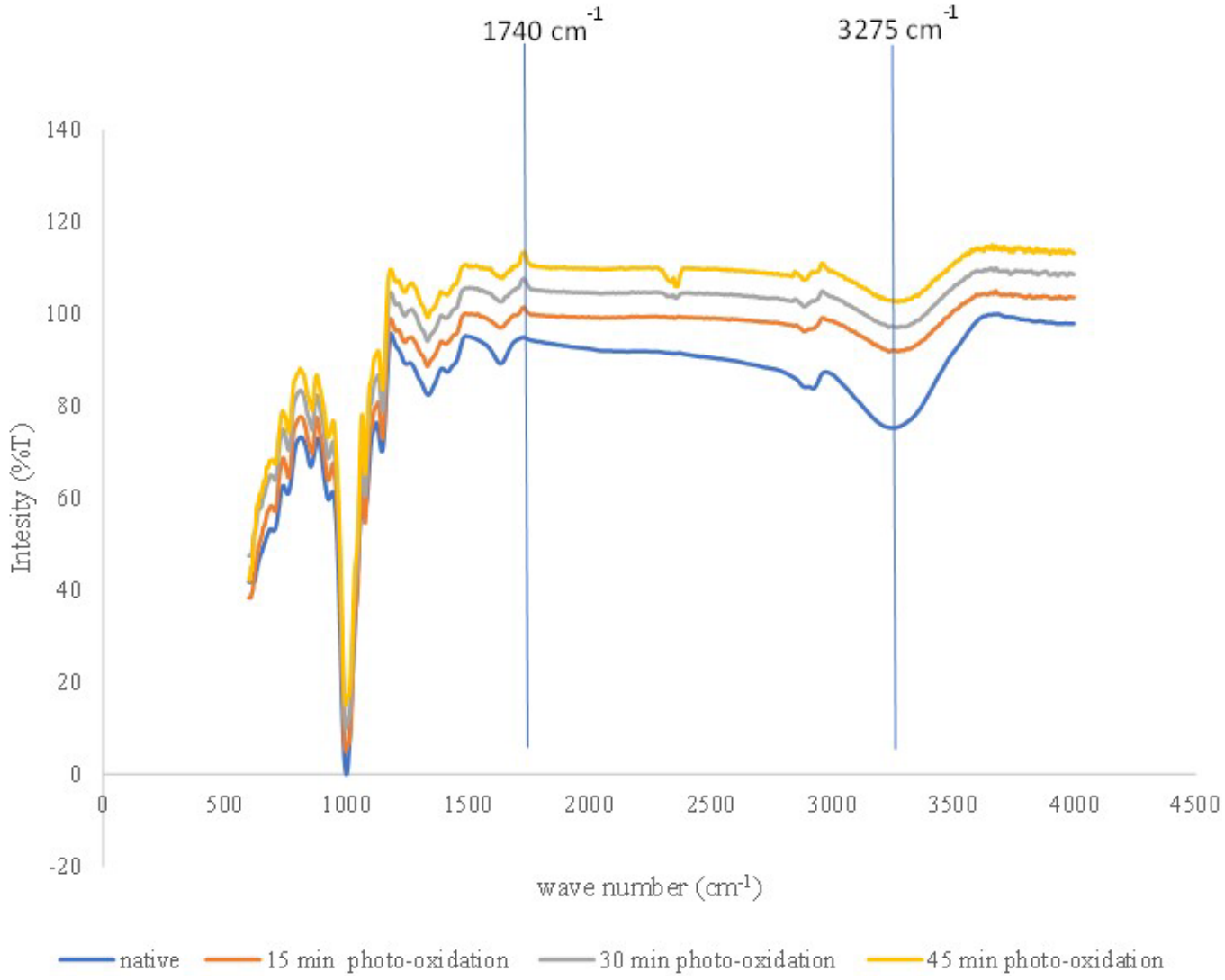

Figure 2. FTIR patterns of native cassava starch and modification by photo-oxidation.

Table 2. Pasting properties of native and photooxidized cassava starch.

Samples	PV (cP)*	BV (cP)*	FV (cP)*	SV (cP)*	Peak time (min)*	PT (°C)*
Native starch	6,982.00 ± 14.00^{d}	3,890.00 ± 17.00^{b}	4,564.00 ± 243.00^{d}	1,472.00 ± 240.00^{c}	3.93 ± 0.00^{c}	73.20 ± 0.05^{c}
Photooxidized starch at exposure times of 15 min	3,442.00 ± 279.00^{c}	2,769.50 ± 543.50^{a}	1,091.00 ± 539.00^{a}	299.00 ± 155.00^{a}	3.63 ± 0.10^{a}	73.15 ± 0.05^{c}
Photooxidized starch at exposure times of 30 min	4,003.00 ± 56.00^{b}	2,337.00 ± 22.50^{a}	2,459.50 ± 22.50^{b}	697.50 ± 41.50^{b}	3.80 ± 0.00^{b}	72.40 ± 0.05^{b}
Photooxidized starch at exposure times of 45 min	4,322.67 ± 173.02^{a}	3,655.50 ± 116.00^{b}	1,353.00 ± 116.00^{a}	488.00 ± 79.00^{ab}	3.60 ± 0.07^{a}	72.30 ± 0.00^{a}

*The same superscript in the same column shows that the samples were not significantly different at 5% significance.

Peak time indicates the time required for flour/starch to reach maximum viscosity. The peak time of photooxidized cassava starch (3.60–3.80 min) tends to be lower compared to the peak time of native cassava starch (3.93 min). The oxidation process also results in a decrease in peak temperature for photooxidized cassava starch (Table 2). The peak temperature of native starch, initially at 73.20°C, decreases to 72.30–73.15°C. A significant decrease in peak temperature is observed with prolonged reaction time. This reduction in pasting temperature is attributed to the weakening and disintegration of starch molecules during the oxidation process (Lawal, 2004). This decrease is attributed to the increased hydration capacity of starch molecules, leading to a reduction in the energy required for the gelatinization process (Wang & Wang, 2003; Zaidul et al., 2007).

3.4 XRD

Overall, there are no significant differences in the X-ray diffractogram between the native and the photooxidized cassava starch (Figure 3). The identity peaks of a typical C-type crystalline of cassava starch were observed at 2θ of 15°, 17°, 18.1°, and 23.3° (Segura & Sira, 2003). Relative crystalline values of the treated samples were significantly lower than that of the native sample. Moreover, longer photooxidation time resulted in the lower relative crystallinity values of the treated samples. These results emphasized that the photooxidation process partly destroyed the crystalline structure of cassava starch. During photooxidation, the generated free radical species act as a molecular scissor destroying randomly the crystalline and amorphous structure (Craig et al., 2005).

3.5 Morphological structure

The morphological forms of starch are related to its functional properties, and the scanning electron microscopy results, one of the morphological assays, are displayed in Figure 4. For 15 min photooxidation, the granule size of the cassava starch is round and slightly elongated. This shape was in line with previous findings, as noted by Hasmadi et al. (2021) and Satmalawati et al. (2020).

The granule shapes in treatments with photooxidation for 15 min, 30 min, and 45 min are different from the native cassava starch, while the starch granules treated with photooxidation for 25 min and 30 min showed rough surfaces and porous shapes. The starch granules treated by photooxidation for 45 min become irregular shapes and roughest compared to other treatments. This is consistent with previous research stating that the ozonized treatment irregular starch form. The prolonged oxidation time led to an intensive erosion of the starch structure. These results supported the solubility, WAC, and OAC that increased with the change of the SEM profile; moreover, the crystallinity proved this result that the lower crystallinity meant a change from a crystalline to a noncrystalline structure.

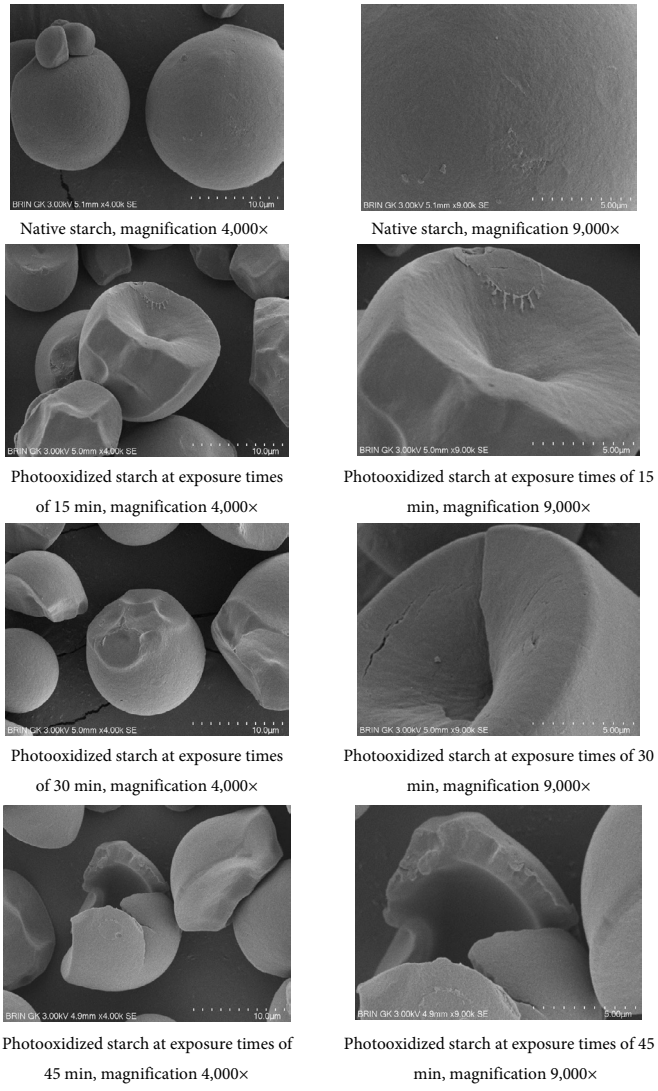

Figure 4. SEM observation results of native cassava starch and photooxidized starch at exposure times of 15, 30, and 45 min at 4,000× and 9,000× magnification.

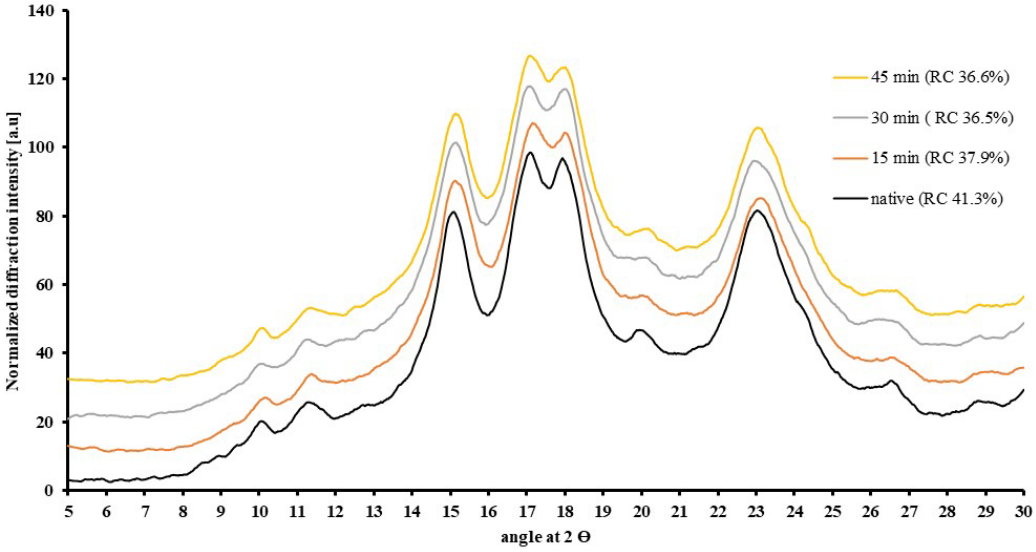

Figure 3. XRD pattern of native and photooxidized cassava starch and relative crystallinity (RC).

4 CONCLUSION

Photooxidation exposure time affects the physicochemical, functional, structural, and morphological properties of cassava starch. The longer exposure time to photooxidation causes an increase in carboxyl content, amylose, solubility, WAC, OAC, and L* value. However, it causes a decrease in the pH value, SP, pasting properties, and relative crystallinity. Apart from that, photooxidation also causes the formation of carbonyl groups and damage to granule morphology. This shows that photooxidation is successful in modifying cassava starch and further research is needed for its application.

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