Chemical properties, functionality, and morphology of taro flour modified by H$_2$O$_2$ oxidation and irradiation of UV light

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Abstract. Taro tuber is one type of traditional Indonesian tubers which is potential to be processed into flour. Modification of taro flour using H$_2$O$_2$ oxidation catalysed with UV irradiation can improve the quality of taro flour. This study aims to determine the chemical properties, functionality, and morphology of modified taro flour affected by concentration H$_2$O$_2$ and oxidation-UV irradiation time. The study used a completely randomized design with two factors, hydrogen peroxide concentration (3, 4, and 5 percent) and time of oxidation-UV irradiation (5, 15, 25, and 35 minutes). Taro flour was analysed in terms of carbonyl, carboxyl, and amylose content pasting properties (peak viscosity, final viscosity, and setback, viscosity), baking expansion, crystallinity, and granular morphology. The results show that the higher the hydrogen peroxide concentration and the longer the reaction time caused the increase in the carbonyl, carboxyl, and amylose contents, peak viscosity, final viscosity, and baking expansion. Meanwhile, it caused a decrease in setback viscosity, relative crystallinity, and damaged the morphological structure of flour granules. All these characteristics are recommended for bakery product production.

1. Introduction
Taro is one type of traditional Indonesian tubers. Taro tuber has the potential to be further processed into taro flour. Taro flour has a total starch of 58.5-63%, resistant starch of 33.5-51.4%, dietary fiber of 12.8-14.0%, and phenolic components of 1.2-4.17 g GA/L, as well as a fairly complete mineral content such as Cu, Fe, Zn, Mn, Mg, and K [1]. Starch in taro flour has disadvantages including high retrogradation tendency, low paste stability and resistance, and it contains oxalate which causes irritation to the tissue when consumed [2]. This limits the application of taro flour in the food industries. Therefore, an effort is needed to modify starch in taro flour, one of which is through the oxidation process.

One way for starch oxidation is by using hydrogen peroxide. Hydrogen peroxide is a widely used commercial oxidizer and does not cause harmful by products [3]. Several studies reveal that oxidation using hydrogen peroxide produces a degree of oxidation of less than 2.78% [4]. To increase functional groups in gelatinized starches, metal ions are often used together with hydrogen peroxide, such as Cu
and Fe. However, excessive use of catalysts can cause undesirable colors on starch, while consumers want bright starch and low metal content [4]. Therefore, the use of metals needs to be limited. Thus, it is essential to use a catalyst that does not cause residues, one of which is by ultraviolet (UV) irradiation.

Modification of starch/flour with hydrogen peroxide oxidation and UV irradiation can be affected by hydrogen peroxide concentration, reaction time, slurry concentration, UV intensity, acid addition, and temperature [5–9]. The higher the concentration of hydrogen peroxide and the longer the reaction, the level of oxidation will increase to a certain extent which is characterized by the formation of carbonyl and carboxyl groups [5,7,8,10]. Starch oxidation also affects molecular starch granules, crystalline patterns, morphology, physicochemical and pasting properties of starch, and results in increased baking expansion [3,5,8,11–13]. This oxidized flour is suitable for application in bakery product [14–16], so that, research and development on oxidized flour need to be done, especially for locals tuber flour to expand its utilization.

A recent modification of taro flour using hydrogen peroxide with and without UV irradiation has been done [14,17]. However, there is no study concerning the effect of UV light on the characteristics of taro flour oxidized with hydrogen peroxide in various hydrogen peroxide concentration and UV oxidation-irradiation reaction time treatments. Therefore, we analyzed the modification of taro flour with oxidation and irradiation to determine its effect on the chemical, functionality, and morphology of taro flour in various treatments of hydrogen peroxide concentration and reaction time.

2. Materials and Methods

2.1. Taro flour preparation
Taro flour was made from Bogor’s taro (Colocasia esculenta L.Schoot) tubers from the Bogor region, West Java. The tubers were peeled, cut, and washed to remove their sap. Furthermore, their size was reduced using a slicer. The sliced tubers then soaked in 10% NaCl solution to decrease the oxalate content that can cause itching and irritation of tissue when consumed. After five times of washing, the taro slices were immersed, sliced, and dried. The dried taro slices were then mashed and sieved to produce taro flour.

2.2. Preparation of taro flour for modification of oxidation-irradiation
The taro flour was oxidized using a UV catalyst tool. Taro flour and reverse osmosis water with a ratio of 1:5 were mixed with hydrogen peroxide oxidizer of the 3,4,5% dry weight of taro flour in a UV catalyst tank. The slurry stirred to prevent sedimentation. The slurry was then streamed to a UV irradiation tube for 5, 15, 25, and 30 minutes of irradiation. After the oxidation process and irradiation stopped, the slurries were centrifuged at 3500 g for 7 minutes and washed three times. Furthermore, the obtained flour cake was dried to get the taro flour with a water content of 10–13%.

2.3. Analysis
Chemical analyzes were carried out including amylose content [18], carbonyl content [19], carboxyl content [20], pasting analysis using Rapid Visco Analyzer Tec Master [21], analysis of baking property modified from the Demiate et al. (2000) method [20], crystalline structure with X-ray Diffraction (XRD) [22] with fitting data from tool readings were done using SAXIT software developed by the Synchotron Light Research Institute (SLRI) Thailand, and morphology of starch granules done using scanning electron micrograph (SEM Hitachi SU3500) with voltage acceleration of 5 and 10 kV at 3000 times magnification [23]

3. Results and Discussion

3.1. Carbonyl, carboxyl and amylose contents
The amount of carbonyl group formed is one indication of the oxidation rate that occurs [10]. Table 1 shows that there was an increase in the carbonyl and carboxyl content of modified taro flour compared
to native taro flour, which were significantly different (p<0.05). Increased carbonyl content occurred in the treatment of hydrogen peroxide with 3% and 4% concentrations with oxidation-irradiation time for 5-35 minutes and (0.0335-0.1121%) then the numbers decreased in the treatment of 5% hydrogen peroxide concentration at 25 and 35 minutes of the oxidation-irradiation time (0.0620 and 0.0466%).

Table 1. Carbonyl, carboxyl, and amylose contents of native and modified taro flour affected by H$_2$O$_2$ concentration and reaction time.

| H$_2$O$_2$ Concentration (%) | Oxidation-irradiation time (minute) | *Carbonyl (%) | *Carboxyl (%) | Amylose (%) |
|-----------------------------|-------------------------------------|---------------|--------------|------------|
| Native flour                | 0.0400$^{ab}$                       | 0.0002$^a$    | 13.26$^a$   |
|                            | 0.0335$^{ab}$                       | 0.0336$^a$    | 15.06$^b$   |
|                            | 0.0720$^{ef}$                       | 0.0608$^{de}$ | 15.13$^b$   |
|                            | 0.0988$^{g}$                        | 0.0626$^{de}$ | 15.32$^{bc}$|
|                            | 0.0985$^{f}$                        | 0.0697$^{e}$  | 15.58$^{bc}$|
|                            | 0.0291$^{a}$                        | 0.0494$^{e}$  | 15.57$^{bcd}$|
| 4                           | 0.0685$^{ef}$                       | 0.0609$^{de}$ | 15.57$^{bcd}$|
|                            | 0.1083$^{g}$                        | 0.0642$^{e}$  | 15.94$^{cd}$|
|                            | 0.1121$^{g}$                        | 0.0690$^{e}$  | 15.52$^{bcd}$|
|                            | 0.0510$^{ad}$                       | 0.0625$^{d}$  | 15.86$^{d}$ |
| 5                           | 0.0786$^{f}$                        | 0.0834$^{f}$  | 16.16$^{d}$ |
|                            | 0.0620$^{de}$                       | 0.0717$^{e}$  | 16.05$^{d}$ |
|                            | 0.0466$^{bc}$                       | 0.0522$^{cd}$ | 15.48$^{bcd}$|

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

The increase in carbonyl content is similar to oxidation in rice flour, sagoo and cassava starch [8]. The higher carbonyl content is related to the molecular fragmentation that occurs due to oxidation treatment. In the reaction pathway, the hydroxyl group in the starch molecule will first be oxidized to the carbonyl group then to the carboxyl group [9]. Decreased carbonyl content in taro flour resulting from oxidation can be caused by carbonyl groups formed during the oxidation process to turn into carboxyl groups [9][12].

Table 1 also shows the increase in carboxyl content of taro flour from oxidation-irradiation modification at hydrogen peroxide 3% and 4% with oxidation-irradiation time for 5-35 minutes and at a concentration of 5% at 5-15 minutes oxidation-irradiation (0.0336-0.0834%) then decreased at 25 and 35 minutes oxidation time (0.0717-0.0522%). The increase in carboxyl content in taro flour resulting from oxidation-irradiation can be caused by further oxidation of carbonyl to carboxyl and the decrease in carboxyl content that occurs can be caused by the occurrence of decarboxylation reactions [9].

The amylose content of native taro flour was 13.26% which increased to 15.06-16.16% and was significantly different (p<0.05) after being given oxidation and irradiation treatments. The higher the concentration of hydrogen peroxide and the longer the oxidation-irradiation time used, the higher the amylose content. This phenomenon is caused by the depolymerisation of starch molecules to produce carboxyl groups and amylose breakdown with high molecular weights into polymers with shorter molecular chains in greater numbers [24]. Oxidation would cleave starch molecules in addition to producing carboxyl groups. The cleavage of high molecular weight amylose molecules would yield some amylose that still could be detected in measurement. This phenomenon similar with oxidized starch prepared from corn, sagoo, and cocoyam starch [5,24,25].
Table 2. Pasting properties and baking expansion of native and modified taro flour effected by H$_2$O$_2$ concentration and reaction time.

| Treatment          | Pasting properties | Specific volume (g/ml) | Baking expansion (%) |
|--------------------|--------------------|------------------------|----------------------|
| H$_2$O$_2$ concentration (%) | Oxidation-irradiation time (min) | Peak viscosity (cP) | Final viscosity (cP) | Setback viscosity (cP) | | |
| native             | -                  | 4043.50$^a$           | 3372.50$^a$         | 1120.00$^b$        | 2.91$^a$               | - |
| 5                  | 4497.00$^b$        | 3490.67$^a$           | 1098.67$^a$         | 3.15$^a$           | 8.23 |
| 3                  | 15                 | 4893.00$^{cd}$        | 3883.50$^{bc}$      | 1213.00$^{ab}$     | 3.67$^b$               | 26.05 |
| 25                 | 4975.00$^{cde}$    | 3780.00$^b$           | 1001.50$^{ab}$      | 3.72$^{b}$         | 27.81 |
| 35                 | 5217.50$^{fgh}$    | 3931.00$^{bc}$        | 881.50$^{ab}$       | 3.73$^{b}$         | 28.11 |
| 5                  | 4639.00$^b$        | 3727.33$^b$           | 1028.50$^{ab}$      | 3.54$^{b}$         | 21.64 |
| 4                  | 15                 | 5058.00$^{def}$       | 3876.22$^{bc}$      | 1029.50$^{ab}$     | 3.77$^{b}$             | 29.51 |
| 25                 | 5014.00$^{cde}$    | 3740.50$^b$           | 1104.33$^{ab}$      | 3.81$^{b}$         | 30.74 |
| 35                 | 5321.00$^{gh}$     | 3962.67$^{bc}$        | 1012.33$^{ab}$      | 3.82$^{b}$         | 31.28 |
| 5                  | 4872.33$^c$        | 3811.00$^b$           | 1056.67$^{ab}$      | 3.58$^{b}$         | 23.14 |
| 15                 | 5234.00$^{fgh}$    | 4080.33$^{cd}$        | 930.67$^{ab}$       | 4.24$^c$           | 45.78 |
| 25                 | 5383.00$^h$        | 4217.00$^{f}$         | 869.50$^{ab}$       | 4.19$^c$           | 43.87 |
| 35                 | 5144.33$^{efg}$    | 3893.67$^{bc}$        | 845.33$^a$          | 4.02$^c$           | 37.95 |

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

3.2. Pasting properties and baking expansion

The higher the hydrogen peroxide concentration and the longer the oxidation-irradiation time, the higher the peak viscosity (4497-5383.00 cP). The value was significantly different from the peak viscosity of native taro flour (p<0.05) and it was significantly different for each treatment (p<0.05). Similar trends were shown by the value of final viscosity. The final viscosity of taro flour from oxidation-irradiation modification ranged from 3490.67 to 4217.00 cP. The highest peak viscosity and final viscosity values of the taro flour were obtained in the treatment of 5% hydrogen peroxide concentration and 25 minutes oxidation-irradiation time (5383.00 and 4217.00 cP). The high peak viscosity is due to the presence of carbonyl and carboxyl groups that trigger swelling in the granules and also shows the presence of crosslinked starch [26]. These cross-links could stabilize the swollen granules and overcome the negative impacts of minor depolymerization [27].

The increase in viscosity when the starch paste is cooled is called setback viscosity. The setback viscosity of the taro flour was 1120 cP, which was higher than the setback viscosity reported by Kaushal et al., 2012 [21], which was 487 cP. This suggests that taro flour has a high retrogradation tendency. The oxidation-irradiation treatment decreased the value of the taro flour setback viscosity compared to native taro flour but these values were not significantly different (p>0.05), except at 5% hydrogen peroxide concentration treatment with 35 minutes oxidation-irradiation time, the lowest viscosity setback was 845.33 cP. Table 2 shows that the higher the concentration of hydrogen peroxide and the longer the oxidation-irradiation time, the lower the back viscosity. The decrease in retrogradation tendencies is caused by the presence of carbonyl and carboxyl groups in more bulky starch molecules than hydroxyl groups, which tends to keep the amylose chains separate so as to inhibit the retrograde process [28]. Less set back viscosity also suggesting that amylose could not form a strong gel network due to depolymerization of starch structure, resulting in a weakened granule organization [27].

Table 2 shows the specific volume and the baking expansion property of native taro flour and taro flour modified by oxidation-irradiation. Native taro flour has a specific volume of 2.91 ml/g which increased significantly in oxidation-irradiation treatment (p <0.05) to 3.15-4.24 ml/g. The higher the concentration of hydrogen peroxide and the longer the UV oxidation time, the higher the specific volume of the taro flour modified with oxidation-irradiation. The highest specific volume of the taro flour was
obtained in the 5% concentration treatment with 15 minutes oxidation-irradiation time, which was 4.24 ml/g or the baking expansion increased by 45.78% compared to the specific volume of native taro flour (2.91 ml/g). Oxidation with hydrogen peroxide with UV irradiation causes the oxidation process to be more effective and produces more carbonyl and carboxyl groups so that the starch binds more water and expands more during the baking process [12]. Increased starch hydration capacity contributes to the increased water-holding capacity in starch molecules; thus, affecting water evaporation and internal pressure during the baking process [5,8]. Meanwhile the increase in baking expansion is caused by the formation of the structure of the amorphous matrix by hydrogen bonds [13].

3.3. X-ray diffraction
Observation of crystallinity of taro flour using X-Ray Diffraction and observations under Scanning Electron Micrograph (SEM) was carried out on native taro flour and modified taro flour with oxidation-irradiation with the highest baking expansion (sample treated with 5% hydrogen peroxide and 15, 25, and 35 minutes oxidation-irradiation time).

![Diffractogram of native and modified taro flour](image)

Figure 1. Diffractogram of native and modified taro flour effected by H$_2$O$_2$ concentration and reaction time.

The taro flour diffraction curve (Figure 1.) shows that taro flour has a reflection of 2θ at 15°, 17°, 18°, and 23° so that it has a type A crystallinity and has a crystalline area of 40.7%. A high 2θ reflection at 15°, 17°, 18°, and 23° indicates a starch with type A crystallinity [29]. Taro flour with 5% hydrogen peroxide concentration and 15, 25, and 35 minutes reaction time display the same X-ray diffraction pattern with the pattern of native taro flour with strong peaks at 15, 17, 18, and 23° (2θ). This means that the oxidation modification process with hydrogen peroxide and UV irradiation does not change the crystallinity pattern of taro flour but slightly decreases the relative crystallinity. This is similar to the results of oxidation on corn starch [28]. The unchanging crystalline pattern of starch is caused by the oxidation process that does not enter the starch granules to change the crystal structure [4]. The decrease in the relative crystallinity of taro flour treated with hydrogen peroxide with 25 and 35 minutes oxidation-irradiation time to 39.8 dan 40.1% can be caused by the slight degradation of the crystalline structure on taro flour due to the increased degree of oxidation along with increasing oxidation time.
3.4. Granules morphology
Figure 2 shows that native taro flour starch granules have irregular polygonal shapes and appear to form together like clusters. The samples treated with UV oxidation have significant differences from the native taro flour granules. The treated taro flours appear to be fragmented into particles with a smaller size and they are more distributed (do not form clusters). This is similar to oxidized taro flour modified by hydrogen peroxide only [14]. In cassava starch [12] the oxidation process causes the granular external structure to be imperfect which can be seen from the surface of starch granules that becomes more coarse compared to the native starch. This does not occur in taro flour granules.

Figure 2. Taro flour granules on SEM observation.
(a. native taro flour ; b. 5% H\textsubscript{2}O\textsubscript{2}, 15 minutes; c. 5% H\textsubscript{2}O\textsubscript{2}, 25 minutes; d. 5% H\textsubscript{2}O\textsubscript{2}, 35 minutes)

4. Conclusions
The oxidation-irradiation process in taro flour produced taro flour with characteristics of: increased carbonyl (0.0335-0.1121%), carboxyl (0.0336-0.0834%), and amylose content increased to 15.06-16.16%. The higher hydrogen peroxide concentration and the longer reaction time resulted in an increased peak viscosity (4497-5383.00 cP), final viscosity (3490.67-4217.00 cP), baking expansion (8.23-45.78%), and decrease setback viscosity (1213.00-845.33 cP). Relative crystallinity decreases without changing the crystalline structure of starch, and it causes the granules that seemed fragmented into smaller and more distributed particles without damage the external structure of the granule.

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