

THE EFFECT OF OXIDATION ON SAGO STARCH AND ITS APPLICATION AS EDIBLE FILM

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ABSTRACT

THE EFFECT OF OXIDATION ON SAGO STARCH AND ITS APPLICATION AS EDIBLE FILM. The oxidation reaction with hydrogen peroxide in sago starch can influence the proximate test value, physicochemical properties and edible film properties. The hydrogen peroxide formula for starch modification is 1, 2, 3 and 4%. The presence of hydrogen peroxide increases the physicochemical properties of sago starch so that the quality of modified sago starch is better than pure sago starch. Glycerol is used to improve film flexibility in the manufacture of edible coatings. The characterization of edible film includes water content, contact angle and elongation. Edible film from modified sago starch shows lower water content compared to pure sago starch films. The oxidation effect of H₂O₂ on edible film results in changes in the hydrophilic properties including the contact angle. Edible film of pure sago starch shows a lower contact angle value. The hydrophilic nature of edible film decreases in the presence of hydrogen peroxide. The high hydrophobicity of the modified sago starch film is caused by the role of carboxyl groups in the starch molecule chain.

Keywords: Oxidation, Hydrogen peroxide, Physicochemical properties, Edible film, Hydrophobicity

ABSTRAK

PENGARUH OKSIDASI PADA PATI SAGU DAN APLIKASINYA SEBAGAI EDIBLE FILM.

Reaksi oksidasi dengan hidrogen peroksida pada pati sagu dapat mempengaruhi nilai uji proksimat, sifat fisikokimia serta sifat *edible film*. Formula hidrogen peroksida untuk modifikasi pati adalah 1, 2, 3, dan 4%. Adanya hidrogen peroksida, meningkatkan sifat fisikokimia dari pati sagu sehingga kualitas pati sagu yang dimodifikasi lebih baik daripada pati sagu murni. Gliserol digunakan untuk meningkatkan fleksibilitas *film* pada pembuatan edible coating. Karakterisasi *edible film* meliputi kadar air, *contact angle* dan elongasi. *Edible film* dari pati sagu yang dimodifikasi menunjukkan kadar air yang lebih rendah dibandingkan dengan film pati sagu murni. Efek oksidasi oleh H₂O₂ pada *edible film* menghasilkan perubahan pada sifat hidrofilik termasuk *contact angle*. *Edible film* pati sagu murni menunjukkan nilai *contact angle* yang lebih kecil. Sifat hidrofilik dari *edible film* menurun dengan adanya hidrogen peroksida. Hidrofobisitas yang tinggi dari *film* pati sagu yang dimodifikasi disebabkan oleh peran gugus karboksil yang ada pada rantai molekul pati.

Kata kunci: Oksidasi, Hidrogen peroksida, Sifat fisikokimia, *Edible film*, Hidrofobisitas

INTRODUCTION

Starch is the second most abundant biopolymer after cellulose found in high level plants, renewable, and available in all parts of the world. Sago is one of several tropical plants that contain a very large amount of starch

found in the stem with a capacity of up to 300 kg and its productivity reaches four times greater than rice. [1].

In terms of quality, natural sago starch has several disadvantages such as inconsistent viscosity or varied

paste profiles, unusual odor, and faded colors. Limitations in terms of resistance to shear and low temperature and having a high tendency towards retrogradation become constraint factors in terms of consumer acceptance and application. [2].

To overcome the limitations in terms of functionalities to be acceptable by consumers, a modification of sago starch is carried out by using H₂O₂ which is an oxidizing agent. Oxidation will convert the hydroxyl group on starch to carboxyl, oxidation will also break down the glycosidic bonds on starch molecules. The modified starch with oxidation method is widely used in the food and nonfood industries where film formation and adhesion properties are needed. [3].

Edible films are renewable packaging. The advantages of an edible film include the diffusion of oxygen and water vapor into the material needed, inhibiting decay by microbes and its safety for consumption. Plasticizer is needed to make an elastic continuous layer. The type of plasticizer used is glycerol. [4].

The focus of this research is to find out the characteristics of pure starch and oxidizedly modified starch for later edible films made from pure sago starch and oxidizedly modified starch and to characterize the mechanical properties of edible film from pure starch and modified starch.

EXPERIMENT METHOD

Materials and Equipment

Sago starch (Ambon), distilled water of 30% hydrogen peroxide, pure N-hexane K₂SO₄ glycerol amylose (Merck, Darmstadt, Germany), formaldehyde, PP indicator, NaOH ethanol pro analyst iodine potassium iodide acetic acid HCL (Merck, Darmstadt, Germany). JEOL JEM 1400 Transmission Electron Microscope (TEM) for microstructure analysis.

Procedure

Oxidised Modification of Sago Starch with Hydrogen Peroxide: Pure sago starch is oxidized using Hydrogen Peroxide with concentrates of 1, 2, 3 and 4% according to the method carried out by Catarina et al (2016) [5]. 100 grams of pure sago starch were dissolved in distilled water to produce starch suspensions. Then it was stirred using a stirrer at room temperature then H₂O₂ was added. After that, stir for 120 minutes. Next, cleaning was done 4 times using distilled water. Then it was filtered and dried at a temperature of 500 °C for 48 hours. The dried starch powder produced was smoothed using a 100 mesh filter.

The Making of Edible Film: The making edible film was conducted using pure sago and modified sago which contains the optimal concentration of H₂O₂. The

sample was weighed 5 grams and then mixed with 100 mL of distilled water. Then stirred for 30 minutes at a temperature of 600 °C. Glycerol was added as a plasticizer with a concentration of 1% (v/v). 10 grams of sample was poured on a petri dish with a diameter of 100 mm. The sample was dried overnight at a temperature of 450 °C. [6].

Characterization

Characterization of pure sago and sago which has been modified by oxidation includes water content, fat content and ash content based on Nielsen method, S (2017) [7], amylose content, and protein content based on the Northrop (1926) method [8], solubility and swelling power were carried out based on Senanayake et al (2013) [9] method, gelatinization temperature was carried out based on Akpa et al (2012) [10] method, and the determination of carboxyl groups was carried out based on Biduski et al (2017) [11] method. Modified functional group analysis of pure cassava starch and sago using FTIR at wave numbers 4000-400 cm⁻¹. Analysis of functional group of pure cassava starch and modified sago used FTIR at wave number 4000-400 cm⁻¹. Morpho-logical analysis of pure cassava starch and modified sago used a scanning electron microscope (SEM) HITACHI SU3500. Water content analysis on edible film was carried out by the method (Moreno et al., 2017) [6], contact angle with Ballesterosa et al (2018) [12] method, and SEM, tensile strength and elongation were carried out on edible coating.

RESULTS AND DISCUSSION

The results of the proximate analysis of pure sago and modified sago are presented in Table 1. Based on the water content data shown in Table 1, the water content will decrease with increasing H₂O₂ concentration so that it is in accordance with the predetermined specification standards. The water content of pure sago starch has not met SNI standard 3729:2008 Sago Flour and also Codex Stan 301R-2011 which requires that the maximum permissible water content in sago flour is 13% b/b. [13,14].

High water level can cause sago starch to be easily damaged because it is susceptible to attack by fungi and other microflora. [5]. (Catarina et al., 2016). Minimum standard value for ash content is <7. Pure and modified sago meet the specification standards. Ash content

Table 1. Results of proximate test of pure and modified sago.

Proximat test	Sago native starch	H ₂ O ₂			
		1%	2%	3%	4%
Moisture content (%)	55.67	10.26	9.95	9.17	8.48
Ash content (%)	1.04	0.56	0.57	0.58	0.78
Protein content (%)	0.0125	0.025	0.043	0.043	0.012
Lipid content (%)	2.41	1.41	1.30	1.07	0.92

increases with increasing H_2O_2 concentration. Protein levels have a minimum standard value of 9.9. Protein levels and the fat content of pure and modified sago tend to decrease with increasing concentration of H_2O_2 .

The ability of starch to hold water is known by using the swelling power test. Swelling power value of pure and modified sago starch can be seen in Figure 1.

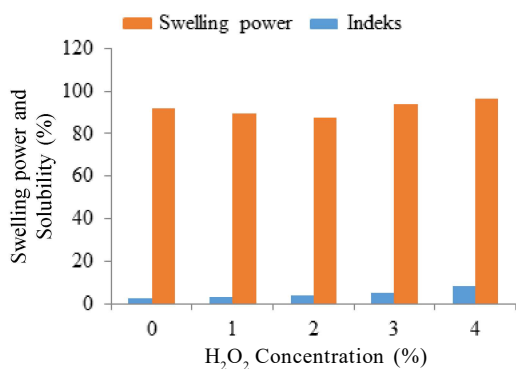


Figure 1. Swelling power solubility index.

The swelling power value increases as H_2O_2 level increases. It increases due to the integration of starch granules during the oxidation process. The increase in swelling power value is possible due to the porous structure of starch granules (SEM results can be seen in figure 7) because of the introduction of a carboxyl group that makes the starch absorb more water and can hold the absorbed water. [15].

The percentage of starch released after the swelling of starch granules can be seen using solubility index testing. The solubility value of sago starch tends to increase. Increased solubility can function in the encapsulation of food products and food additives. Solubility of modified starch tends to increase due to depolymerization and weakening of the internal structure of starch granules. [16].

Pure sago starch requires high temperatures to experience gelatinization. The presence of water forms hydrogen bonds between OH groups of sago starch with water molecules. This bond replaces the hydrogen bond between the pure starch chain which results in bond dissociation. [17].

The decrease in gelatinization temperature (Figure 2) due to oxidation effects can weaken the granular structure and disintegration occurs during the modification process. Oxidation of sago starch is one method that modifies the rheological properties of sago starch which results in depolymerization and weakens granular structure. [18]. As a result, the gelatinization temperature decreases due to the influence of the entry of hydroxyl group into the polymer chain and makes the bond unstable. [17]. Amylose content of pure and modified sago starch is presented in Figure 3. The amylose content increases when H_2O_2 is added.

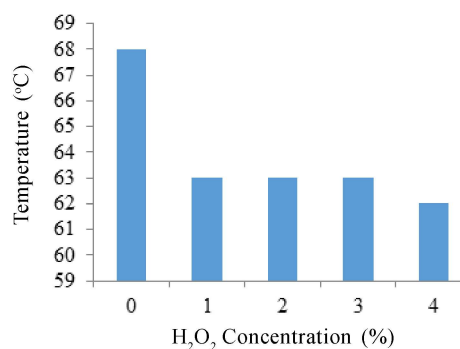


Figure 2. Gelatinization temperature of sago starch

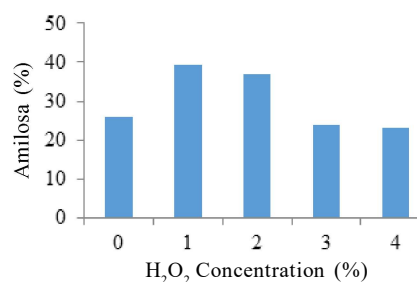


Figure 3. Amylose content of sago starch.

The increase in amylose content is related to the depolymerization of molecular bonds of sago starch where the length of sago starch bonds breaks the chain so that there is a shortening of the sago molecular bonds and is divided into a large number. [5]. Oxidation of sago starch causes degradation of amylose and amylopectin molecules in α -1,4-glycosidic bonds. Oxidation with H_2O_2 results in an increase in the amylose value of starch. [19].

During the oxidation process, the OH group in the sago starch molecule will be oxidized to carbonyl and carboxyl. The sequential reaction to the OH group in the sago starch molecule in the initial stage will be oxidized to the carbonyl group then into the carboxyl group. [20].

The carboxyl group content (Figure 4) increases with increasing H_2O_2 added. This is due to further oxidation of the carbonyl group to the carboxyl group. [19]. The reaction that occurs between pure sago starch and H_2O_2 as follows.

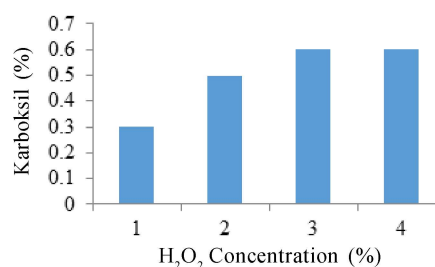


Figure 4. The carboxyl group content in sago starch.

Based on Figure 5, OH group will be oxidized by H_2O_2 to form a compound containing the carboxyl group. Based on the graph in Figure 4, the highest value of the carboxyl group is obtained with a value of 0.599% with H_2O_2 concentrations of 3 and 4%. The value of carboxyl group obtained has proven that a complete oxidation reaction occurs by H_2O_2 . [21].

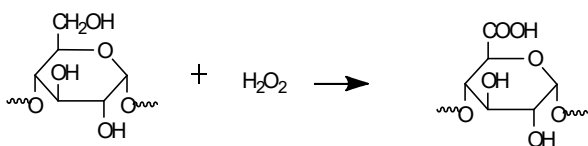


Figure 5. Reaction of native sago and H_2O_2 .

Transition temperature (T_o , T_p and T_c) and enthalpy energy (ΔH_g) are related to gelatinization temperature of pure and modified sago starch presented in Table 2. The onset temperature (T_o) of oxidizedly modified starch is higher than pure sago starch.

Table 2. The enthalpy energy are related to gelatinization temperature of pure and modified sago starch

Sample	H_2O_2 (%)	Gelatinization Temperature			Enthalpy (ΔH_g) (J/g)
		T_o ($^{\circ}C$)	T_p ($^{\circ}C$)	T_c ($^{\circ}C$)	
Native sago	0	40	103	120	11.2
Modified of sago	1	60	90	120	1.5

To increase of modified sago starch was due to depolymerization in the amorphous region which served to destabilize the crystals. After the amorphous region is degraded, the crystal domain is destroyed due to the destabilizing effect. Therefore, gelatinization of modified sago starch produced occurred at a higher temperature than pure sago starch. [22].

Modified sago starch has lower T_p than pure sago starch. This is due to a decrease in crystallinity. In the oxidation treatment process, the amorphous region of the modified starch is destroyed first then the crystalline region is partially oxidized causing instability in the crystal and amorphous regions which causes a lower melting temperature after the oxidation treatment [23].

Enthalpy energy from modified sago starch is lower than pure sago starch. This is due to oxidation by H_2O_2 which weakens the granules of starch and possibly also due to partial degradation of the sago starch molecule. As a result, less energy is needed to gelatinize modified starch. [22].

Thermal stability of pure and modified sago were analyzed using TGA and DTA analysis. TGA and DTA curves of pure and modified sago starch are shown in Figures 6 and 7.

From these data, it is known that the thermal degradation of pure and modified sago has similarities. This shows that the modified sago does not significantly

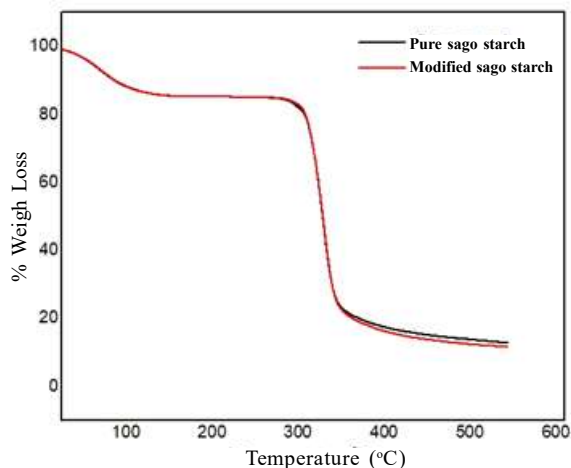


Figure 6. TGA graph of pure and modified sago starch.

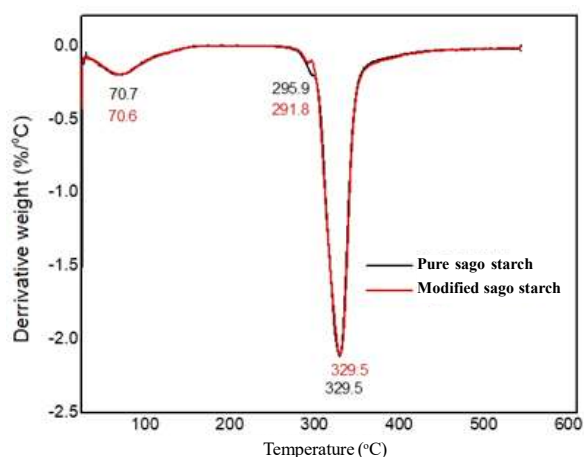


Figure 7. DTA graph of pure and modified sago starch

change the thermal stability. Pure and modified sago each decomposed at 295.90C and 291.80C with a residual weight of 85%. The small change in thermal stability between pure and modified sago is due to the small content of the carboxyl group obtained at modified sago which is equal to 0.599%. Therefore, it does not significantly change the constituent structure of modified sago.

The morphology of pure and modified sago starch was analyzed using SEM to see granules from both. From Figure 8 it can be seen that there has been a stretch of granular bond due to the influence of H_2O_2 . This stretch of bond is very clearly seen because in pure sago starch, the formed granular bond still looks very strong. The presence of H_2O_2 as an oxidizing agent results in depolymerization and weakening of the internal structure of starch granules caused by changes in the group in the modified starch. As a result, physicochemical properties of starch may be better than pure sago starch [26].

Pure sago starch has large granules with many rough surfaces. This happened due to the treatment carried out during the study. Pure sago starch is treated

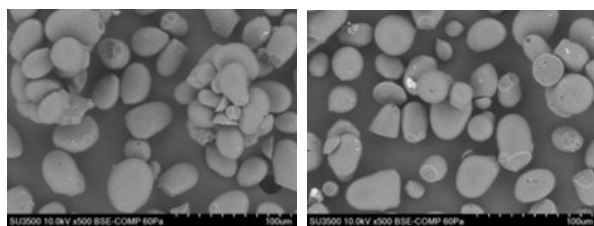


Figure 8. SEM analysis of (a). pure sago starch and (b). modified sago starch.

by grinding when the preparation process is done so that the surface of pure sago starch has large granules with many rough surfaces. On the other hand, modified sago starch has small grains with a smooth surface due to nongrinding process. [27].

Characterization of Edible Film

The water content of edible film from pure and modified sago starch can be seen in Table 3. The water content of edible film from pure sago starch is higher than that of modified one. The presence of glycerol in the film formula causes an increase in the hydrophilic nature of the film. Whileas edible films from modified sago starch have a lower water content. The starch molecule chain is broken by H_2O_2 so that the water content value decreases. The water content of the film affects the ability to absorb water in a film. The high water content causes the quality of the film to have a shorter service life due to bacterial interference. [28].

Table 3. Moisture content of edible film.

Sample	Glycerol (%)	Mo	M	Mc
Edible film of Native sago	1	41.4802	41.3591	0.29
Edible film of modified sago	1	50.9304	50.7973	0.26
	1.5	51.5333	51.3486	0.36
	2	48.6136	48.2868	0.67
	2.5	49.3323	48.9662	0.73

The quality of edible film can be seen from the hydrophilic nature of a film that can be determined using contact angles testing. The results of the testing from pure and modified sago can be seen in Figure 9.

Pure sago starch has a smaller contact angle value than modified sago starch. The contact angle value of pure sago starch containing 1% glycerol was 41.92, while

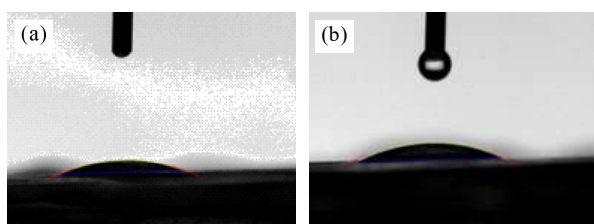


Figure 9. Contact angle (a). Pure sago starch (b). Modified sago starch.

that of modified sago starch containing 1% glycerol was 45.47. The effect of oxidation by H_2O_2 on edible film results in changes in the contact angle that significantly increases the contact angle value. [12].

The results obtained showed that the hydrophilic properties of sago starch correlated with the contact angle value. The high contact angle value of modified sago starch caused a decrease in the hydrophilic properties of modified sago starch films and improved hydrophobic properties. Likewise, the opposite is true of pure sago starch. The hydrophobic nature of the edible film of the modified sago starch results in better film quality because the film is impermeable to water so it has very good quality when used as a food coating material. [12].

Lengthening is defined as the percentage of changes in film length when the film is pulled out. Films made from starch are easily damaged. Increasing the concentration of the material, will increase the matrix formed, so the film will become strong. However, increasing the concentration of the material also causes a decrease in the ratio of glycerol as a plasticizer to starch, resulting in a decrease in the elongation of the film when exposed to the force, which then causes the film to break easily. The elongation value of modified sago starch is 2.68 mm and pure sago starch of 2.32 mm. [21]. In natural sago starch, the granule bond is still very strong so that the formed film becomes rigid and starched. This causes the value of film elongation from natural sago starch smaller than the film of modified sago starch.

CONCLUSION

Modified sago starch has better quality than pure sago starch reviewed from physicochemical properties. The highest carboxyl group value was obtained at H_2O_2 concentration of 4% which proved that OH group of sago starch was completely oxidized. Enthalpy energy from modified sago starch has decreased due to the oxidation effect of H_2O_2 which can weakens the starch granules. The molecular weight of pure sago starch is lighter, making it easier to decompose.

Edible films from modified sago starch have a lower water content than those from pure sago starch. The water content of the film affects the ability to absorb water in a film. The high water content causes the quality of the film to have a shorter service life due to bacterial interference. The hydrophilic nature of edible film decreases due to the presence of H_2O_2 . Modified sago starch has a film that is more hydrophobic than pure sago starch. The elongation value of modified sago starch was 2.68 mm.

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REFERENCES

- [1]. H. Ehara." Potency of sago palm as a carbohydrate resource for strengthening the food security program." *Japanese Studies Journal Special Issue:Regional Cooperation for Sustainable Future in Asia*. 2012.
- [2]. J. Li and S. Nie. "The functional and nutritional aspects of hydrocolloids in foods." *Food Hydrocolloids*, vol 53, pp. 46-61. 2016.
- [3]. H. Xiao, Q. Lin, G. Liu and F. Yu. "A Comparative Study of the Characteristics of Cross-Linked, Oxidized and Dual-Modified Rice Starches." *Molecules*, vol 17, pp. 10946-10957. 2012.
- [4]. F. Saleh, A. Nugroho dan Juliantama, R. "Pembuatan Edible Film dari Pati Singkong sebagai Pengemas Makanan." *Teknoin*, vol 23, no. 1, pp. 43-48. 2017.
- [5]. S. Catarina, A.Kumoro, R. Ratnawati, dan S. Retnowati. "Modifikasi pati sukun (*artocarpus altilis*) dengan teknik oksidasi menggunakan hidrogen peroksida tanpa katalis." *Teknik*, vol 37, no. 1, pp. 32-40. 2016.
- [6]. O. Moreno, J. Cardenas, L. Atarés and A. Chiralt. "Influence of starch oxidation on the functionality of starch-gelatin based active films." *Carbohydrate Polymers*, vol 178, pp. 147–158. 2017.
- [7]. S. Nielsen." Food Analysis." 5th Ed. New York, NY: Springer, 2017. [AOAC] Association of Official Analytical Chemists. (2006). Official Methods of Analysis of The Association of Official Agriculture Chemists. Virginia (US): AOAC International.
- [8]. J.H. Northrop." A convenient method for the formol titration." Insitute for Medical Research, 1926.
- [9]. S. Senanayake, A. Gunaratne, K.K.D.S. Ranawera and A. Bamunuarachchi. "Effect of heat moisture treatment conditions on swelling power and water soluble index of different cultivars of sweet patato (*Ipomea Batatas* (L). Lam) starch." *ISRN Agronomy*. Hindawi Publishing Corporation, pp. 14. 2013.
- [10]. Akpa, J. Gunorubon, Dagde and K. Kekpugile. "Modification of Cassava Starch for Industrial Use." *International Journal of Engineering and Technology*, vol 2, pp. 913-919. 2012.
- [11]. B. Biduski, F. Silva, W. Silva, S. El Halal, V. Pinto, A. Dias and E. Zavareze. "Impact of acid and oxidative modifications, single or dual, of sorghum starch on biodegradable films." *Food Chemistry*, vol 214, pp. 53-60. 2017.
- [12]. F. Ballesterosa, M. Cerqueira, J. Teixeira and S.Mussatto."Production and physicochemical properties of carboxymethyl cellulose films enriched with spent coffee grounds polysaccharides." *International Journal of Biological Macromolecules*, vpl 106, pp. 647-655. 2018.
- [13]. Badan Standarisasi Nasional. 2008. Tepung Sagu . SNI 3729:2008. Jakarta (ID): BSN
- [14]. E. Fitria. "Sifat Fisikokimia Pati Sagu Termodifikasi dengan Metode Oksidasi Menggunakan Natrium Hipoklorit." Institut Pertanian Bogor, 2017.
- [15]. L.M. Fonseca, J.R. Goncalves, S.L.M.E. Halal, V.Z. Pinto, A.R.G. Dias, A.C. Jaccques and E.R. Zavareze." Oxidation of potato starch with different sodium hypochlorite concentrations and its effect on biodegradable films. " *LWT Food Science and Technology* pp. 714-720. 2015.
- [16]. P. Parovuori, A. Hamunen, P. Forssell, K. Autio, K.Poutanen, Espoo, and Raiio. "Oxidation of Potato Starch by Hydrogen Peroxide." *Starch*, vol 47, pp. 19-23. 1995.
- [17]. A. Ali, T. Ahmed, I. Wani and F. Masoodi. "Comparative study of the physico-chemical properties of rice and corn starches grown in Indian temperate climate." *Journal of the Saudi Society of Agricultural Sciences*, vol 15, no. 1, pp. 75-82. 2016.
- [18]. Z. Iqbal and D. Ciptaningtyas. "Designing and Experimenting Semi-automatic Green Grass Jelly Squeezer." *International Journal on Advanced Science, Engineering and Information Technology*, vol 8, no. 4. 2018.
- [19]. N. Vanier, S. El Halal, A. Dias and E. Zavareze. Molecular structure, functionality and applications of oxidized starches: A review. " *Food Chemistry*, vol 221, pp. 1546-1559. 2017.
- [20]. E. Tethool, A. Jading, dan B. Santoso. " Pengaruh konsentrasi hydrogen peroksida dan irradiasi ultraviolet terhadap sifat fisikokimia dan baking expansion pati sagu. Prosiding InSINas, 2012.
- [21]. W. Rachmayanti dan E. Kusumo. "Karak terisasi Antimicrobial Film dari Ekstrak Kedelai dan Tapioka sebagai Bahan Pengemas Makanan." *Indonesian Journal of Chemical Science*, vol 4, no. 3. 2015.
- [22]. P.S. Hornung, A.G. Granza, C.S. de Oliveira, M.E. Lazzarotto. "Schnitzler Study of the effects of ultraviolet light and sodium hypochlorite solutions on properties of cassava starch granules." *Food Biophysics*, vol 10, pp. 368-374. 2015.
- [23]. S. Wang dan L. Copeland. "Effect of acid hydrolysis on starch structure and functionality: A review." *Critical Review Food Science*, vol 55, pp, 1081-1097. 2015.
- [24]. Zhuang, F. Tao, Y. Cui. "Anti-degradation gelatin films crosslinked by active ester based on cellulose." *RSC Advnaced*, vol 5, pp. 52183–52193. 2015.
- [25]. Zuo, J. Gu, L. Yang, Z. Qiao, H. Tan and Y. Zhang. "Preparation and characterization of dialdehyde starch by one-step acid hydrolysis and oxidation." *International Journal of Biological Macromolecules*, vol 103, pp. 1257–1264. 2017.

- [26]. L.H. Garrido, E. Schnitzler, M.E.B. Zortea, T.S. Rocha and I.M. Demiat. "Physicochemical properties of cassava starch oxidized by sodium hypochlorite." *Journal of Food Sciences and Technology*, vol. 51, no. 10, pp. 2640-2647. 2012.
- [27]. S. Pietrzyka, T. Fortuna, L. Juszczyk, D. Gałkowska, B. Małgorzata, Aczkowicz M. Łabanowska and M. Kurdzielb. "Influence of amylose content and oxidation level of potato starch on acetylation, granule structure and radicals' formation." *International Journal of Biology and Macromolecules*, vol 106, pp. 57-67. 2018.
- [28]. F.M. Pelissari, M.M. AndradeMahecha, P.J.d. Sobral, F.C. Menegalli. "Comparative study on the properties of flour and starch films of plantain bananas (*Musa paradisiaca*)."
Food Hydrocolloids, vol 30, no. 2, pp. 681-690. 2013.



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