Improvement of physicochemical characteristics of *kano* white yam flour (*Dioscorea rotundata*) through fermentation process using SBM.3D and SBM.4A lactic acid bacteria

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**Abstract.** This research aimed to determine the effects of the fermentation process using SBM.3D and SBM.4A lactic acid bacteria (LAB) isolates on the physicochemical characteristic of *kano* white yam flour. This research used a factorial Completely Randomized Design (CRD) consisting of two factors. The results showed that the interaction of LAB types and LAB concentrations had very significant effects on the viscosity (30.10 cP), swelling power (11.40 g/g), water solubility index (21.91%), and the acidity (pH=6.20) of white yam flour. Based on SEM and FTIR analysis showed that liberation of starch from within the granules which can result in changes chemical properties, viscosity, and morphology of the resulting starches. We conclude that the physical properties of flour improved after fermentation process using SBM.3D and SBM.4A LAB isolates.

1. **Introduction**

The high use of wheat flour in food products so far has caused dependence on wheat flour. Wheat flour consumption in Indonesia in 2017 was 2.586 thousand tons or increased by 5.52% [1]. This increase in consumption of flour every year is due to the increase in demand of the wheat flour based-food products such as bread, noodles, biscuits and cookies.

One of the efforts to overcome the dependence of wheat flour is to use local food ingredients as an alternative to wheat flour, namely white yam (*Dioscorea rotundata*) or known as *kano* yam by the people of Wakatobi. [2] stated that the carbohydrate content of *kano* yam is 40.61% (dmb) so that *kano* yam can be used as flour. According to [3] *kano* flour has low gluten levels so that it can be consumed by children with special needs (autistic). However, there are some physicochemical properties that need to be modified in order to improve the characteristics of the flour.

The disadvantages of *kano* flour include having low water solubility, loaf volume, and viscosity. This causes limited use of native starch from *kano* yam in food industries [4]. One way that can be done for starch modification is by fermentation using lactic acid bacteria (LAB). Bimo and
Widhyastuti [5], reported that modification with LAB on Asiatic bitter yam (Dioscorea hispida) reduced HCN levels and increased the content of protein to 10.03%, fat 0.48%, and total acids 1.67 mg/g. Adnan et al. [6] characterized 10 LAB isolates from fermentation of wakawondu red rice rinsing water and found 4 superior isolates. Of the 4 superior isolates, 2 isolates of LAB SBM.3D and SBM.4A are probiotic agents. The SBM.3D and SBM.4A isolates were selected based on the relatively large starch and casein degradation ability and the ability to grow at low pH.

Lactic acid fermentation in tubers is an effort to modify tuber cells through fermentation using lactic acid bacteria (LAB). LAB produces enzymes and organic acids which degrade some of the starches into shorter-chain polymers thereby improving the functional properties of flour [7].

In this research, kano white yams (Dioscorea rotundata) was modified using LAB from wakawondu red rice washing water. This modified flour was expected to have better physicochemical properties and organoleptic value so that it can be applied to the manufacture of functional foods that are beneficial to health.

2. Materials and methods

2.1 Materials

The main ingredients are kano yam, lactic acid bacteria (LAB) with isolate codes of SBM.3D and SBM.4A were taken from the Plant Protection Laboratory of the Phytopathology Unit of the Faculty of Agriculture, Halu Oleo University, Kendari. LAB growth media was MRS-Agar (Himedia). The chemicals for physicochemical analysis were LABfroad reagent, Nelson-Smogy reagent (Merck), Arsenomolybdat reagent (Merck), Pb acetate (Merck), glucose standard solution (Merck), AgNO3 0.002 N (Merck), n-hexane (Merck), Bovine serum albumin (Sigma), and Biuret reagent (Merck).

2.2 Methods

2.2.1 Rejuvenation of lactic acid bacteria (LAB) SBM.3D and SBM.4A. LAB rejuvenation process was done using the spread plate method. A single colony with LAB characterization was scraped back on MRS-Agar media. After that, incubation was performed for two days at room temperature to obtain pure LAB isolates.

2.2.2 Kano white yam modification process through fermentation using LAB isolates of SBM.3D and SBM.4A. Kano yam was washed, cut into small pieces evenly with a thickness of ± 3 cm, and put into a sterilized glass jar. Fermentation was a solid-state fermentation where inoculums (SBM.3D and SBM.4A) were added in three different concentration (OD 0.50; 0.75 and 1.00) on sliced kano yam with an inoculum volume of 10% of the test material. Incubation was done at 35 °C for 24, 48, and 72 hours. After fermentation, kano yam was washed with sterile water and processed into flour.

2.2.3 Physicochemical properties of modified kano white yam

2.2.3.1 Viscosity. Viscosity analysis was carried out using the Oswald viscometer (Oswald method) [8]. This test was done by measuring the time required by a suspension of kano yam flour to flow from the Oswald viscometer until it reached the stop point.

2.2.3.2 Swelling power and water solubility index (WSI). Swelling power was the ratio between the weight of sludge left in the centrifuge with the sample dry weight. While the water solubility index (WSI) was the percentage weight of water-soluble starch [9].

2.2.3.3 pH. The pH value was measured using a Jeneway 3505 pH meter. Before use, the pH meter was calibrated using a buffer of pH 4 and pH 7.
2.2.4 Morphology analysis with SEM. The sample was placed on an aluminum plate that had two sides. The sample was then coated with a gold layer under vacuum to make a conductive sample. Starch morphology was observed using a scanning electron microscope (SEM Philips XL30) and images were taken at a potential acceleration of 20 kV [10].

2.2.5 FTIR. A certain amount of flour was mixed uniformly with KBr to form pellets using manual pressing equipment (Shimadzu, Tokyo, Japan). The FTIR spectrum was made using the ABB MB3000 FTIR spectrophotometer (Clakuadeset Scientific, Northampton, UK) with a DTGS detector in the infrared region (4000 - 400 cm\(^{-1}\)) with a resolution of 4 cm\(^{-1}\) [11].

2.3 Data analysis
Data on physicochemical characteristics of kano yam flour were analysed with Analysis of Variance (ANOVA) of a Completely Randomized Design (CRD) experiment. If the results of the ANOVA had F value> F table at \(\alpha = 0.05\), the treatment had a significant effect on the response variable and the test was followed by Duncan's multiple range test (DMRT) with a 95% confidence level.

3. Results and discussion

3.1 Physicochemical properties of modified kano flour

3.1.1 Viscosity. The highest viscosity value (30.10 cP) (Table 1) was obtained from H2B2 treatment, which used SBM.4A isolate at the concentration of OD = 0.75. The increase in the viscosity of modified kano yam flour was caused by LAB activity. [12] reported that the viscosity value of modified Asiatic bitter yam increased by 7.87-15.90 cP after fermentation with LAB from wakawondu red rice rinsing water. A similar study by [13] showed that the viscosity value of Dioscorea rotundata flour which was fermented by L. acidophilus and B. longum increased to 10.92 cP. An increase in the viscosity of kano yam flour was due to the LAB activity that produced extracellular amylase enzymes that cause starch degradation during fermentation by oxidizing amorphous portions and simultaneously hydrolyzing amylose and amylopectin [14] causing swelling of the starch granules [15].

3.1.2 Swelling power. Swelling power of modified kano flour of treatment H2B2 (SBM.4A isolate at the concentration of OD = 0.75) was the highest (11.40 g/g) (Table 2). This condition was caused by cell liberation that occurred during fermentation. [12] reported that the value of swelling power of modified Asiatic bitter yam that was fermented with LAB from rinsing water of wakawondu red rice rose by 9.46 g/g. Cell liberation that occurs during fermentation releases amylose and amylopectin from the starch granule, which increases the water binding sites during gelatinization. The increase in the value of swelling power and solubility is determined by the activity of microbes that degrade starch resulting in shorter starch chains, which absorb more water.

The swelling power is also affected by the size and strength of the micellar tissue bonding, amylose molecular structure, and amylose content. Swelling power will increase with increase in amylopectin content (branched chain structure) and with decrease in amylose content [16]. Amylopectin is in the amorphous region of starch granules. Amorphous areas are tenuous and less dense, making it easily absorbs water [17]. The more amylopectin in starch, the wider the amorphous area, which leads to a greater water absorption. According to [18] starch properties during gelatinization are influenced by the ratio of amylose and amylopectin. Amylopectin content plays a role in the dough development, while the amylose inhibits it.
The highest WSI value (23.91%) (Table 2) was obtained from H2B2 treatment, which used SBM.4A isolate at the concentration of OD = 0.75. An increase in the water solubility index is caused by the effect of the LAB fermentation process. [12] reported that the solubility index value of modified Asiatic bitter yam (Dioscorea hispida Denst) flour increased by 8.77% after the yam tubers underwent fermentation using LAB isolates from wakawondu red rice rinsing water.

According to [19], fermentation will increase the value of water solubility index, because during the fermentation process, starches are hydrolyzed into shorter chains, which are highly hydrophilic and easily bind to water. According to [20], amylase is one of the factors that influence the solubility value because amylose is soluble in water. Furthermore, an increase in the amorphous portion will cause an increase in the solubility, because this portion is more easily destroyed in hot water compared to the crystalline part of a starch.

3.1.4 pH. Based on Table 1, the interaction of isolate types and LAB concentration on the pH value of modified kano yam flour was 6.14 in the H2B3 treatment (SBM.4A, OD = 1.00). This shows that the higher the concentrations of LAB and the longer the fermentation time, the resulting pH value decreased. A similar study by [12], pH value of fermented Asiatic bitter yam flour by LAB from wakawondu red rice rinsing water decreased to 5.94. Reduction of pH value during fermentation is due to the acid compounds that are produced by LAB [21]. The microbes will produce amylase enzymes that degrade starch resulting monosaccharides that will undergo metabolic processes to produce organic acids, especially lactic acid [19].

3.2 Starch morphology analysis with SEM
Changes in the shape of starch granules can be seen in the results of the Scanning Electron Microscope in Figure 1. Modified kano starch granules looks denser than the native kano starch granules. The granules of modified kano starch also split into several parts. In the presence of extracellular amylolytic enzyme, the starch granules are partially hydrolyzed on the surface, resulting in hollow starch granules. This allows the liberation of starch from within the granules which can result in changes in the chemical properties, viscosity, and morphology of the resulting starches.

| Code  | Treatment                      | Viscosity (cP) | Swelling Power (g/g) | WSI (%) | pH       |
|-------|--------------------------------|----------------|----------------------|---------|----------|
| H1B1  | (LAB SBM.3D OD : 0.50)         | 29.77±0.02     | 9.37±0.01            | 23.13±0.02 | 6.20±0.02 |
| H2B1  | (LAB SBM.4A OD : 0.50)         | 29.28±0.03     | 11.17±0.09           | 23.29±0.03 | 6.22±0.03 |
| H3B1  | (LAB Combination OD : 0.50)    | 29.44±0.03     | 9.54±0.02            | 23.19±0.01 | 6.65±0.01 |
| H1B2  | (LAB SBM.3D OD : 0.75)         | 30.04±0.02     | 10.54±0.01           | 23.62±0.01 | 6.16±0.01 |
| H2B2  | (LAB SBM.4A OD : 0.75)         | 30.10±0.02     | 11.40±0.04           | 23.91±0.03 | 6.20±0.03 |
| H3B2  | (LAB Combination OD : 0.75)    | 29.64±0.03     | 10.32±0.01           | 23.66±0.02 | 6.63±0.02 |
| H1B3  | (LAB SBM.3A OD : 1.00)         | 29.13±0.02     | 9.55±0.02            | 23.30±0.02 | 6.15±0.02 |
| H2B3  | (LAB SBM.4A OD : 1.00)         | 30.07±0.03     | 10.11±0.07           | 23.65±0.02 | 6.14±0.02 |
| H3B3  | (LAB Combination OD : 1.00)    | 29.47±0.03     | 9.59±0.01            | 23.60±0.03 | 6.41±0.03 |

The numbers followed by different letter notations show significant differences based on the 0.05 DMRT of 95% confidence level. H1: SBM.3D; H2: SBM.4A; H3: Combination of SBM.3D and SBM.4A, B1: OD 0.50; B2: OD 0.75; B3: OD 1.00.
3.3 FTIR analysis

The FTIR spectrum in Figure 2 are the absorption band of both native and modified kano yam flour. The samples spectrum shows the same absorption at wavelengths of 2931 cm\(^{-1}\) (aliphatic \(-\text{CH}_2\) groups) which is strengthened by the presence of CH bonds at wavelengths of 1419 cm\(^{-1}\) and 1373 cm\(^{-1}\), 1651 cm\(^{-1}\) (stretching COO groups) and 1157 cm\(^{-1}\) (C - C stretching). Sifts in the FTIR spectrum due to the fermentation occurred in the OH stretching group with a wavelength of 3425 cm\(^{-1}\) for native flour and 3410 cm\(^{-1}\) for modified flour and also in the glycosidic COH group with a wavelength of 995 cm\(^{-1}\) for native flour and 1018 cm\(^{-1}\) for modified flour. Based on this identification it can be seen that the modification of functional groups occurred due to the interaction of LAB in the kano yam [22].

4. Conclusion

The best fermentation time in making modified kano yam flour was 48 hours using SBM.4A LAB isolate with OD = 0.75. The interaction of LAB types and concentrations significantly affected the physicochemical characteristics of modified flour. DMRT test results (P ≤ 0.05) revealed significant differences in organoleptic and nutritional values of modified kano yam flour compared to the native flour without fermentation. This result give recommendation to produce kano white yam flour with fermentation using SBM.4A LAB isolate on the optimum condition to get a better physicochemical flour properties quality.

References

[1] Statistik Pertanian [Agriculture Statistics] 2017 Konsumsi tepung terigu [Wheat flour consumption] (Indonesia: Kementerian Pertanian [Ministry of Agriculture])
[2] Alinnor I J and Akalezi C O 2010 Proximate and mineral compositions of Dioscorea rotundata (white yam) and Colocasia esculenta (white cocoyam) J. Pakistan Nutr. 9 (10) pp 998-1001

[3] Lionora G, Dewi D R S, and Rahaju D E S 2013 Analisis kelayakan bisnis kue muffin dari tepung uwi [Feasibility analysis of muffin cake business from uwi flour] J. Widya Teknik 12 (1) pp 92–102

[4] Kantouch and Taufik S 1998 Gelatinization of hypochlorite oxidized maize starch in aqueous solutions J. Starch 2 (3) pp 114-9

[5] Bimo R H S and Widyastuti N 2016 Pengaruh fermentasi bakteri asam laktat terhadap sifat fisikokimia tepung gadung modifikasi (Dioscorea hispida) [Effect of lactic acid bacteria fermentation for physicochemical properties of modified yam flour (Dioscorea hispida)] Jurnal Litbang Industri 6 (1) pp 61-72

[6] Adnan N S, Wahyuni S and Khaeruni A 2017 Pengujian sifat amilolitik dan proteolitik dari isolat bakteri asam laktat (BAL) hasil fermentasi air cucian beras merah (Oryza nivara) kultivar wakawondu [Evaluation of amylolytic and proteolytic properties of lactic acid bacteria (LAB) isolates from cultivation of red rice (Oryza nivara) wakawondu] Jurnal Sains dan Teknologi Pangan 2 (5) pp 759-69

[7] Salim E 2011 Mengolah singkong menjadi tepung mocaf [Processing cassava into mocaf flour] (Yogyakarta: Andi Offset)

[8] Jati B M E, Karyono and Supriyatin 2010 Penyertaan nilai viskositas terhadap indeks bias pada zat cair bening [Inclusion of the viscosity value of the refractive index in the liquid] Berkala Fisika 13 (4) pp 119-24

[9] Senanayake S, Gunaratne A, Ranawera K K D S and Bammunuarachchi A 2013 Effect of heat moisture treatment conditions on swelling power and water soluble index of different cultivars of sweet potato (Ipomea batatas (L) Lam) starch ISRN Agronomy pp 1-4

[10] Ashri M S M, Yusof, Jamil M S, Abdullah A, Yusoff S F M, Nasir M and Lazim A M 2014 Physicochemical characterization of starch extracted from malaysian wild yam (dioscorea hispida dennst.) J Food Agriculture 26 (8) pp 652-8

[11] Zhu G, Sheng L and Tong Q 2014 Preparation and characterization of carboxymethyl-gellan and pullulan 410 blend films Food Hydrocolloids 35 pp 341-7

[12] Sidupa H E 2019 Sifat Fisikokimia Tepung Gadung (Dioscorea Hispida Denst) Term modifikasi Dengan Menggunakan Isolat Bakteri Asam Laktat (Sbm.3d Dan Sbm.4a) Asal Wakawondu Dan Aplikasinya Pada Pembuatan Mie Basah [The Physicochemical Properties of Yam Flour (Dioscorea Hispida Denst) were Modified Using Lactic Acid Bacteria (Sbm.3d and Sbm.4a) From Wakawondu and the Applications in Noodles] (Kendari: Universitas Halu Oleo)

[13] Andriyani T, Utami R and Widowati E 2013 Kajian penggunaan tepung uwi putih (Dioscorea rotundata) dalam pembuatan minuman simbiotik terhadap karakteristik fisikokimia, sensori, dan total bakteri probiotik [Study of white yam flour (Dioscorea rotundata) using in producing of symbiotic beverages toward physicochemical characteristics sensory, and total probiotic bacteria] Jurnal Teknologi Hasil Pertanian 6 (1) pp 51-8

[14] Putri W, Marseno D and Cahyanto 2012 Isolasi dan karakterisasi bakteri asam laktat amilolitik selama fermentasi gowol, makanan tradisional Indonesia [Isolation and characterization of amylolytic lactic acid bacteria during growol fermentation, an Indonesian traditional food] Jurnal Teknologi Pertanian 1 (13) pp 52-60

[15] Yeni G, Silfia S, Wilsa H and Tri W 2018 Pengaruh waktu hidrolisis dan konsentrasi HCl terhadap karakteristik pati termodifikasi dari bengkuang (Pachyrhizus erosus) [Effect of hydrolysis time and HCl concentration on modified starch characteristics of jicama (Pachyrhizus erosus)] Jurnal Litbang Industri 8 (2) pp 53-60

[16] Srichuwong S, Takashi M, Sunarti T C and Naoto I 2005 Starches from different botanical sources I: contribution of amylopectin fine structure to thermal properties and enzyme digestibility Carbohydrate Polymers 60 (4) pp 529-38
[17] Haryadi 2006 Teknologi Pengolahan Beras [Rice Processing Technology] (Yogyakarta: Gadjah Mada University Press)

[18] Fredriksson H, Silverio J, Anderson R, Eliasson A C and Aman P 1998 The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches Carbohydrate Polymers 35 pp 3-4 119-34

[19] Pusparani and Yuwono S S 2014 Pengaruh fermentasi alami chips ubi jalar (Ipomoea batatas) terhadap sifat fisik tepung ubi jalar [Effect of natural fermentation in chips of sweet potato (Ipomoea batatas) against physical properties of wheat sweet potato] Jurnal Pangan Dan Agriindustri 2 (2) pp 59-69

[20] Pukkahuta C, Suwannawat B, Shobsngob S and Varavinit S 2008 Comparative study of pasting and thermal transition characteristic of osmotic pressure and heat moisture treated corn starch Carbohydrate Polym 72 pp 527-36

[21] Sidabutar A R, Feliatra and Dahliaty A 2015 Uji Aktivitas Antimikroba Bakteriosin Dari Bakteri Probiotik Yang Diisolasi Dari Udang Windu (Penaeus Monodon Fabricus) [Test Of Bacteriocin Antimicrobial Activity Of Probiotic Bacteria Isolated From Tiger Shrimp (Penaeus Monodon Fabricus)] (Pekanbaru: Universitas Riau)

[22] Wahyuni S, Ansharullah, Saefuddin, Asranudin and Holilah 2017 Physico-chemical properties of wikau maombo flour from cassava (Manihot esculenta crantz) Journal of Food Measurement and Characterization 11 pp 329-36

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